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LINKAGE RELATIONS OF THE GOLDFOIL FACTOR FOR RESISTANCE TO MILDEW IN BARLEY [1]

By FRED N. BRIGGS, *associate agronomist*, and ERNEST H. STANFORD, *junior agronomist, California Agricultural Experiment Station*

INTRODUCTION

Seven different genetic factors for resistance to race 3 of mildew (*Erysiphe graminis hordei*) in barley (*Hordeum vulgare*) have been discovered in the writers' work with this disease (*7*).[2] Six of these factors have either occurred singly in varieties or have been set up in hybrid lines. Two, the Algerian factor Ml_a and the Kwan factor Ml_k, are linked (*2*). The others appear to be independent. Since all seven linkage groups have been established in barley (*6*), the linkage groups to which these seven mildew-resistant factors belong may be ascertained. Linkage relations of the Goldfoil factor are now available.

Incidental to this study, data were accumulated on a factor pair for red vs. green stem color that has not heretofore been reported. Linkage relationships were established for this factor also.

MATERIALS AND METHODS

Briggs and Barry (*1*) reported that the resistance of Goldfoil was due to a single dominant factor, Ml_g, and pointed out that this factor was independent of six-row vs. two-row and long vs. short-haired rachilla, characters available in the cross between Goldfoil and Atlas, and known to belong to linkage groups I and V, respectively. Since these findings were reported Goldfoil has been crossed with susceptible varieties carrying markers for four of the remaining groups. The cross with Nepal 595 was investigated first because it was segregating for two factor pairs each in groups III and IV. Group 3 was marked by hulled vs. naked, *Nn*, and by one of a complementary factor pair for blue vs. white aleurone, Bl_1bl_1, recently reported by Myler and Stanford (*4*). Group IV was marked by hooded vs. awned, *Kk*, and by the other complementary factor pair for blue vs. white, *Blbl*.

Goldfoil is resistant to mildew, is hulled, awned, and white, but carries the *Bl* factor for blue aleurone. Under favorable light conditions it develops red pigment in the stems. Nepal 595 is susceptible to mildew, is naked, hooded, and white, but has the Bl_1 factor for blue. In contrast to Goldfoil it has green stems.

As in previous experiments, the mildew tests were made in the greenhouse with race 3 of the fungus. Mildew classifications were based on the reaction of F_3 progeny, which were grown in greenhouse benches and which in most cases were represented by 25 to 30 plants. Every tenth row was seeded to Atlas as a check. The plants were inoculated in the three-leaf stage by dusting with spores from diseased plants grown for that purpose. The F_3 rows were classified as resistant,

[1] Received for publication March 27, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 5.

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segregating, or susceptible. Since this factor for mildew resistant is dominant, a segregating row indicated a resistant F_2 plant and is so considered in the calculations.

When light intensity was sufficient to cause the red stem color to develop, the F_3 rows were also classified for this character. Homozygous red and segregating rows were listed as red.

The cross-over values and their probable errors were all taken from Immer's tables (*3*).

EXPERIMENTAL RESULTS

A total of 795 F_2 plants of Goldfoil×Nepal 595 were grown in the field in 1939–40 and classified at harvest time for hooded vs. awned, hulled vs. naked, and blue vs. white aleurone. Of these, 770 were tested for mildew reaction in F_3 in the greenhouse during 1940 and 1941, while 25 lines were discarded either because of insufficient seed or because the population was too much reduced by damping-off. It soon became apparent that the Goldfoil factor for mildew resistance was linked with hooded vs. awned and consequently with the blue vs. white factor pair belonging to group IV. Resistance, accordingly, was independent of the factors represented by group III. Only the data involving factors in group IV will be presented in connection with mildew resistance.

The single contrasting characters all conform to expectations on the basis of factors previously assigned to them. There were 562 hooded: 208 awned, where 577.5:192.5 were expected on the basis of a single factor (*6*). This gives a P value greater than 0.2. As pointed out above, Myler and Stanford (*4*) found the blue aleurone color to be due to complementary factors. There were 412 blue:358 white, where 433.1:336.8 were expected on the basis of the 9:7 ratio, giving a P value between 0.1 and 0.2. Finally, there were 588 mildew resistant: 183 mildew susceptible, where 578.25:192.75 were expected on the basis of a single factor, which gives a P value greater than 0.3.

Table 1 shows the data involving hooded vs. awned and resistance vs. susceptibility.

TABLE 1.—*The F_2 segregation of the characters listed from a cross of Goldfoil ($kk\ Ml_gMl_g$)×Nepal 595 ($KK\ ml_gml_g$) with expected numbers based on independence and on a cross-over value of 18.77 percent*

Characters	Observed number	Expected number on the basis of a 9:3:3:1 ratio	χ^2 on the basis of a 9:3:3:1 ratio	Expected number on the basis of a cross-over of 18.77 percent	χ^2 on the basis of a cross-over of 18.77 percent
Hooded resistant	387	433.1	4.907	391.8	0.059
Hooded susceptible	175	144.4	6.484	185.7	.616
Awned resistant	201	144.4	22.185	185.7	1.261
Awned susceptible	7	48.1	35.119	6.8	.006
Total	770	770.0	68.695	770.0	1.942
P value			Very low		>0.3

In table 1 the observed numbers for the character combinations deviate significantly from the numbers expected on the basis of the 9:3:3:1 ratio. A cross-over value of 18.77±2.33 percent was indicated by the data. The numbers expected on the basis of this value agree satisfactorily with those obtained, giving a $P>0.3$.

Table 2 shows the data regarding blue vs. white aleurone and resistance vs. susceptibility to mildew.

TABLE 2.—*The F_2 segregation of the characters listed from a cross of Goldfoil (Bl_1Bl_1 $blbl$ Ml_gMl_g) × Nepal 595 (bl_1bl_1 Bl Bl ml_gml_g) with expected numbers based on independence and on 26.31 percent crossing over*

Characters	Observed number	Expected number on the basis of a 27:9:21:7 ratio	χ^2 on the basis of a 27:9:21:7 ratio	Expected number on the basis of a cross-over of 26.31 percent	χ^2 on the basis of a cross-over of 26.31 percent
Blue resistant	289	324.8	3.946	298.7	0.315
Blue susceptible	123	108.3	1.995	134.4	.967
White resistant	299	252.7	8.483	278.8	1.464
White susceptible	59	84.2	7.542	58.1	.014
Total	770	770.0	21.966	770.0	2.757
P value			Small		>.2

Robertson et al. (*5*) have shown that the *Bl* factor for blue aleurone is linked with hooded with a cross-over value of 22.58±0.82. Since Myler and Stanford (*4*) have shown this gene is present in Goldfoil, it follows that mildew resistance should show linkage with blue aleurone. Obviously the data in table 2 do not conform to independent segregation. The numbers observed agree satisfactorily, however, with those expected on the basis of 26.31 ±5.05 percent recombination.

The data clearly show that the Goldfoil factor pair for resistance vs. susceptibility to mildew is linked with the hooded vs. awned and the blue vs. white aleurone factor pairs, known to belong to group IV. The data are not sufficient to establish the order of these three gene pairs. The linkages reported in this paper have high probable errors because the genes entered the hybrid in the repulsion phase. The most probable cross-over values, with the maximum and minimum that might be expected based on ±3 probable error, with the suggested order, follows:

$$Bl \leftarrow\!\!\text{———}\left\{\begin{array}{c}20.12\\ \mathbf{22.58}\\ \hline 25.04\end{array}\right\}\text{———}\ K \leftarrow\!\!\text{———}\left\{\begin{array}{c}11.78\\ \mathbf{18.77}\\ \hline 25.76\end{array}\right\}\text{———}\ Ml_g$$

$$\longleftarrow\!\!\text{—————}\left\{\begin{array}{c}11.16\\ \mathbf{26.31}\\ \hline 41.46\end{array}\right\}\text{—————}\!\!\longrightarrow$$

As will be seen, the most probable value of the cross-over percentages for *Bl*—*K* and *K*—Ml_g together equals 41.35, which is just within the maximum range expected for *Bl*—Ml_g. In no other case will the sum of any two most probable values fall within the expected range of the third. The most likely order therefore seems to be *Bl*, *K*, Ml_g.

Incidental to the mildew studies, data were accumulated on red vs. green stem color, the genetics of which has not previously been reported. Goldfoil shows considerable red pigment in the stems when grown under favorable light conditions, whereas Nepal 595 produces

no red. Of the 607 plants classified for stem color, 442 were either homozygous red or segregating, while 165 were green. The numbers expected on the basis of the 3:1 ratio are 455.25:151.75, giving a probability greater than 0.2. Red stem will hereafter be designated by the symbol *Rs*.

Hulled vs. naked segregated on the basis of 3:1, which is in agreement with the results of numerous other workers (*6*). There were 448 hulled: 159 naked, where 455.25:151.75 were expected, giving a P>0.3. As pointed out above, blue vs. white aleurone segregated in this cross on the basis of complementary factors. The segregation of the 607 plants under consideration here agreed satisfactorily with that hypothesis, having a *P* value greater than 0.2.

It soon became apparent that red vs. green stem color was associated with hulled vs. naked. Table 3 gives the data involving these characters.

TABLE 3.—*The F_2 segregation of the characters listed from a cross of Goldfoil (NN RsRs)×Nepal 595 (nn rsrs), with expected numbers based on independent segregation and on a cross-over value of 14.5 percent*

Characters	Observed number	Expected number on the basis of a 9:3:3:1 ratio	χ^2 on the basis of a 9:3:3:1 ratio	Expected number based on a cross-over of 14.50 percent	χ^2 based on a cross-over of 14.50 percent
Hulled red	403	341.5	11.075	414.4	0.314
Hulled green	45	113.8	41.594	40.8	.432
Naked red	39	113.8	49.166	40.8	.079
Naked white	120	37.9	177.847	111.0	.730
Total	607	607.0	279.682	607.0	1.555
P value			Very small		>0.3

Obviously the observed numbers do not conform to those expected on the basis of independent segregation, but agree satisfactorily with those expected on the basis of 14.50±1.06 percent crossing over. Thus the factor for red stem color belongs to linkage group III.

As Myler and Stanford (*4*) have shown, the complementary factor for blue aleurone (Bl_1) discovered by them belongs to group III and showed a cross-over percent of 9.88±0.44, with naked. This gene is carried by Goldfoil. Red stem color should be linked, accordingly, with blue aleurone. That this is so may be seen from table 4.

TABLE 4.—*The F_2 segregation of the characters listed from a cross of Goldfoil (Bl_1Bl_1 RsRs blbl)×Nepal 595 (bl_1bl_1 rsrs BlBl), with expected numbers based on independent segregation and on a cross-over of 9.07 percent*

Characters	Observed number	Expected number on the basis of a 27:21:9:7 ratio	χ^2 on the basis of a 27:21:9:7 ratio	Expected number based on a cross-over of 9.07 percent	χ^2 based on a cross-over of 9.07 percent
Red blue	305	256.0	9.379	321.7	0.867
Red white	137	199.2	19.422	133.5	.092
Green blue	20	85.4	50.084	19.7	.005
Green white	145	66.4	93.042	132.1	1.260
Total	607	607.0	171.927	607.0	2.224
P value			Very small		>0.3

The expected numbers based on independent segregation deviate markedly from those observed. Those observed conform, however, to the numbers expected on the basis of 9.07 ± 1.24 percent crossing over.

Although the data are too few to indicate the order of the three gene pairs, they suggest that these genes are in the order of *N*, Bl_1, *Rs*.

SUMMARY

In a cross between Goldfoil, which carried the Goldfoil factor (Ml_g) for resistance to mildew, and which is awned and hulled, with Nepal 595, which carries the contrasting characters, it was found that mildew resistance was linked with hooded (*K*) 18.77 ± 2.33 percent, and with the *Bl* factor for blue aleurone with a cross-over of 26.31 ± 5.05 percent. As Robertson et al. (*5*) had previously shown, *K* and *Bl* are linked with a value of 22.58 ± 0.82 percent. These factors have been assigned to linkage group IV (*6*). Because the factors in this cross enter it in the repulsion phase, the probable errors are relatively high, and the order of the three genes under consideration is not clearly indicated. The order suggested is *Bl*, *K*, Ml_g.

Red stem color in this cross was found to be due to a single factor which was designated as *Rs*. This factor was found to be linked with hulled (*N*), 14.50 ± 1.06 percent, and with a second factor for blue aleurone color (Bl_1) with a cross-over value of 9.07 ± 1.24 percent. These genes have been assigned to linkage group III (*4*, *6*). Here, again, the order of the genes under consideration is not definitely indicated, but the suggested order is *N*, Bl_1, *Rs*.

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SURVIVAL, WEIGHT, AND LOCATION OF EUROPEAN CORN BORERS FEEDING ON RESISTANT AND SUSCEPTIBLE FIELD CORN [1]

By L. H. PATCH [2]

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INTRODUCTION

Certain strains of corn have shown greater resistance to the survival of the European corn borer (*Pyrausta nubilalis* (Hbn.)) than have others. The utilization of this resistance in the practical control of the borer would be facilitated by a better knowledge of the factors responsible for it and of the ways in which they operate. Studies were therefore conducted in 1936 and 1937 near Toledo, Ohio, to determine, if possible, what the factors are and how, where, and when they influence the development of the borer.

One of the most resistant strains of field corn (*Zea mays* L.) yet discovered, single-cross hybrid Ill. Hy × R4, was compared with one of the most susceptible, Ill. A × Ind. TR. Although these hybrids have silked and matured their grain within 1 or 2 days of each other throughout the period of test, from 1932 to 1939, inclusive, every test has shown materially fewer borers surviving in Hy × R4 from a given number of eggs, and has indicated an inherent character for borer resistance. This character was at first thought to be delayed eclosion of the tassel, for in this hybrid the upper leaves remain wrapped around the tassel until nearly time for pollen shedding. Since the additional mature borers surviving in A × TR nearly equaled the additional immature larvae found in the tassels, it was thought probable that the availability of the tassel buds for larval feeding accounted for its relative susceptibility.

The relative survival of borers in the resistant and susceptible hybrids was studied at different stages of plant development. To determine whether the difference between the two hybrids was as great in the absence of tassels as when they are available, the tassels were removed by hand in another experiment. The age of the borers at the time the differentiation in survival occurred, the comparative weights of the borers found within the tassel buds and in other parts of the plants at different ages, and the locations in the plants of the borers throughout their growth were also studied.

EFFECT OF STAGE OF PLANT DEVELOPMENT AND PRESENCE OF TASSELS ON LARVAL SURVIVAL

To assure adequate levels of infestation and a wide range in plant development at time of borer hatching, in 1936 and 1937 corn was

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[2] The writer is indebted for assistance received from the following members of the staff of the Toledo, Ohio, laboratory to W. A. Baker under whose general supervision the work was conducted, to G. T. Bottger and Ralph Mathes for providing egg masses for the infestations, to B. A. App for supervising the counting and weighing of borers from different parts of the plants, to R. T. Everly for assembling and tabulating the data, and to Elmer Beck for the photograph in figure 3.

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planted on 10 or 12 dates, and the plants were infested by hand over an extended period with egg masses produced by moths confined in cages in the laboratory.[3] Results reported in a previous publication [4] indicated that the data obtained under conditions of artificial infestation are comparable with those obtained under natural conditions.

In 1936 the experimental field was divided into 10 strips extending from north to south across the field. In these strips corn was planted, in 3-plant hills 42 inches apart each way, in successive plantings at 2- to 4-day intervals beginning on May 7, the east half of each strip to Hy × R4 and the west half to A × TR. On June 30 the field was again divided into strips at right angles to the first set, or extending from east to west. Beginning at the south end of the field, the first 4 strips of this set marked off plots of 12 hills of each hybrid in each planting and 7 other strips marked off 8-hill plots. The first strip was infested by hand on June 30 with 4 egg masses per plant, hatching within a day, in addition to a light natural infestation. Ten other infestations were made successively in strips toward the north, the last one on July 24.

The stage of plant development, as measured by the number of days from borer hatching to pollen shedding, the average number of mature or nearly mature borers per plant counted from three or four plots for each hybrid, and their ratios, expressed as percentages of the borer-susceptible hybrid, are given in table 1. In figure 1 these percentages have been plotted against the number of days between borer hatching and pollen shedding, and a freehand curve has been fitted to the data.

TABLE 1.—*Effect of stage of plant development, as measured by the interval from borer hatching to pollen shedding, on the number of mature European corn borers in resistant (Hy × R4) and susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1936*

Days from borer hatching to pollen shedding	Borers per plant— In A × TR	Borers per plant— In Hy × R4	Borers per plant— Ratio Hy × R4 to A × TR	Days from borer hatching to pollen shedding	Borers per plant— In A × TR	Borers per plant— In Hy × R4	Borers per plant— Ratio Hy × R4 to A × TR
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
38	8.2	2.5	30	17	4.7	1.5	32
35	8.7	2.6	30	16	6.8	2.5	37
33	9.6	2.5	26	16	8.5	3.5	41
31	8.0	3.5	44	14	6.0	4.1	68
29	8.3	3.3	40	14	12.3	4.7	38
28	8.4	4.1	49	13	11.2	5.8	52
26	7.5	3.1	41	11	14.7	8.1	55
26	6.6	4.0	61	11	15.5	7.9	51
25	5.9	2.5	42	10	10.9	5.8	53
23	8.7	4.3	49	9	10.0	7.7	77
23	4.9	2.5	51	9	18.4	10.3	56
22	8.9	4.5	51	7	14.0	9.2	66
21	4.8	1.6	33	6	23.1	14.6	63
21	7.5	3.4	45	5	16.3	12.4	76
18	6.2	2.2	35	4	19.3	15.6	81
18	8.3	4.6	55	2	25.7	21.3	83
17	10.7	4.8	45				

In both hybrids the number of mature borers per plant on plants on which the borers hatched within 14 days of pollen shedding showed a

[3] PATCH, L. H., and PEIRCE, L. L. LABORATORY PRODUCTION OF CLUSTERS OF EUROPEAN CORN BORER EGGS FOR USE IN HAND INFESTATION OF CORN. Jour. Econ. Ent. 26: 196–204, illus. 1933.

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pronounced increase over the number on plants in an earlier stage of development (table 1).

Figure 1 shows a change also in the ratio between the two hybrids on plants at this stage of development. The ratio increased gradually from 38 to 48 percent as the time between borer hatching and pollen shedding decreased from 38 to 14 days and then increased sharply to 86 percent as the interval decreased to 2 days. The increase in the ratio from 38 to 86 percent shows that hybrid Hy × R4 was much less resistant, relative to A × TR, when the borers hatched just before pollen shedding than when they hatched about 30 days before.

The period within 14 days of pollen shedding had such marked effects on borer survival and the relative number of borers in the two hybrids that the development of the plants within this period was given special study. The tassels of hybrid A × TR came into view

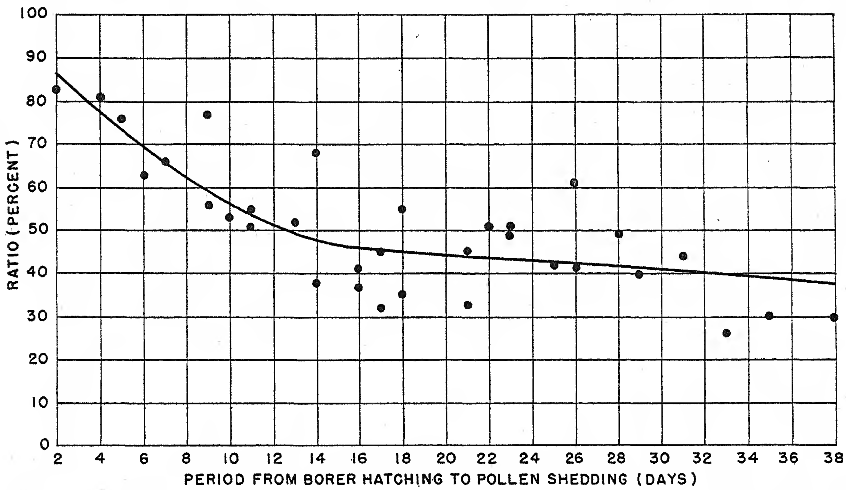

FIGURE 1.—Effect of stage of plant development on the ratio of the number of European corn borers in borer-resistant single-cross hybrid field corn Hy × R4 to the number in borer-susceptible hybrid A × TR, Toledo, Ohio, 1936.

10.3 days before they shed pollen, and the tassels of Hy × R4, although only 1.5 days later in development, came into view 4.2 days before they shed pollen. The tassels, growing up within the enveloping whorl of leaves, were undoubtedly available to the young borers before they came within view of the observer. Data from an experiment in 1937 show that in A × TR 50.5 percent of the borers, 4 days old, were in the tassels 14 to 17 days before the plants shed pollen as compared with 5.1 percent in Hy × R4. Hence the maximum availability of the tassel buds for feeding occurred, at least partly in Hy × R4 and probably wholly in A × TR, within the period when the egg infestations were made that resulted in large increases in the number of mature borers.

To determine whether increases in the level of borers in the two hybrids, both actually and relatively, would occur in the absence of tassels as well as in their presence, 9 pairs of 48-plant plots were planted on May 7, 1937, one plot of each pair to Hy × R4 and the

other to A × TR. All plots were infested with 3 egg masses per plant. On the first 3 pairs of plots the borers hatched, respectively, on July 4, 8, and 12, when the tassels were not visible to the observer, and on the other 6 pairs the borers hatched between July 16, when most of the tassels of A × TR were just visible, and July 24, when many tassels of A × TR were shedding pollen. Before the eggs were placed on the plants of these 6 pairs, the tassels were pulled from one-half of each plot, except the July 16 pair of plots, from which they could not be pulled without mutilating the plants.

The number of borers per plant in each hybrid from the detasseled plants in the plots where the borers hatched on July 19, 20, 21, 23, and 24 were plotted against the date of borer hatch, and freehand curves were fitted to these data (fig. 2). The curve for A × TR is shown as a broken line between July 8 and 19, because the plotted data from the plots where the borers hatched on July 12 and 16 were not used in fitting the curve. The number of borers in the plot where the borers hatched on July 16 was considerably above the level expected from the broken curve in figure 2, because the tassels could not

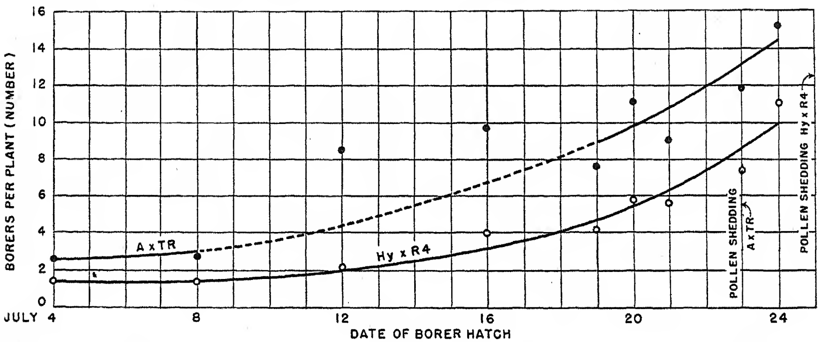

FIGURE 2.—Effect of stage of plant development on the level of the European corn borer population in borer-resistant single-cross hybrid field corn Hy × R4 and borer-susceptible hybrid A × TR when the effect of the tassels was eliminated, Toledo, Ohio, 1937.

be removed. The tassels in the plot of A × TR where the borers hatched on July 12 were beginning to show on July 13. Since 50.5 percent of the borers 4 days old were in tassel buds from 4 to 7 days before the tassels became visible to the observer, the higher-than-expected level of borers in the July 12 plot of A × TR was undoubtedly due to the availability of the tassels.

The trend of the curves in figure 2 indicates that the levels of borers were higher on the more mature plants of both hybrids even though the effect of the tassels had been eliminated. The effect of weather on borer establishment was a possible factor. Records taken in the field show that the weather was especially uniform between July 18 and 25, when the detasseled plants were infested. Between these dates no rain fell and the temperature did not rise above 90° F. A heavy rain of 2.22 inches fell on July 25. However, since there were more borers in the plots of both hybrids where the borers hatched on July 24 than in the other plots, rainfall had no inhibiting effect on borer establishment. The weather between July 3 and 13, when the

infestations were made that resulted in low borer populations, is believed to have been favorable for borer establishment. Stirrett,[5] in studying the effect of four or five rainstorms during the hatching period in each of 3 years, concluded that rain has little effect on larval survival. It is probable, therefore, that the higher borer survival on the more mature plants, indicated in figure 2, was not the result of weather conditions.

From the data at hand the conclusion is drawn that the rate of borer survival increases with advance in plant development at time of borer hatch, at least up to the pollen-shedding stage, and that there is an additional rise in the borer level in those hybrids in which the tassels are more accessible to the young borers. Hybrids with delayed tassel eclosion would probably partly inhibit borer survival if the borers hatched within 14 days of pollen shedding.

The curves in figure 2 indicate that there was an increasingly larger percentage of borers in Hy × R4 as compared with A × TR as the plants approached the pollen-shedding stage. Data taken from the curves show that in the plants where borers hatched July 8 an average of 50.0 percent as many borers were in Hy × R4 as in A × TR, as compared with 52.3 and 68.1 percent in the detasseled plants where borers hatched on July 19 and 24, respectively. Hence it appears that hybrid Hy × R4 lost some of its resistance to the borer relative to A × TR when the borers hatched on plants approaching close to the pollen-shedding stage, even though the effect of the tassels had been removed.

AGE OF BORERS AT TIME OF DIFFERENTIATION IN SURVIVAL

Another experiment similar to that of 1936 was conducted in 1937, which comprised 12 plantings of A × TR and Hy × R4 from May 7 to June 5. Manual infestations with eggs hatching within a day were made on July 8 and 12 on separate plots of each planting. Soon after the eggs hatched, two plants of each hybrid were taken from each planting each work day, the borers in them counted, and their locations in the plants noted. Dissection of plants infested on July 8 alternated with that of plants infested on July 12. It was possible to combine the derived data so that 12-plant samples were made up for 12 different ages of the borers ranging from 4 to 55 days for groups of plants shedding pollen 14, 17, 18, 20, 21, 24, 28, and 32 days after borer hatching. These data were further combined by averaging the 14-, 17-, and 18-day groups from plants in a late stage of development, the 20- and 21-day groups from plants in a midseason stage, and the 24-, 28-, and 32-day groups from plants in an early stage. As a result samples of 36 or 24 plants and a somewhat stabilized ratio of the number of borers in Hy × R4 to those in A × TR were obtained (table 2).

The numbers of borers in the various age groups are given in table 2. In hybrid A × TR the population of borers in the 50.0-day age group of plants in the late stage of development was 58.4 percent, and in plants in the midseason stage 29.1 percent, greater than in plants in

[5] STIRRETT, G. M. A FIELD STUDY OF THE FLIGHT, OVIPOSITION AND ESTABLISHMENT PERIODS IN THE LIFE CYCLE OF THE EUROPEAN CORN BORER-PYRAUSTA NUBILALIS, HBN., AND THE PHYSICAL FACTORS AFFECTING THEM. V. THE SEASONAL CHARACTERISTICS OF FLIGHT, OVIPOSITION AND LARVAL ESTABLISHMENT. THE VARIATIONS AND EFFECTS OF SEASONAL CLIMATE. THE FACTORS CAUSING FLUCTUATIONS IN BORER POPULATIONS. Sci. Agr. 18: 653–683, illus. 1938.

the early stage of development. In the hybrid Hy × R4 there were 39.9 and 21.0 percent more, respectively, in plants in the last two stages of development. Apparently, conditions for borer survival did not improve so much in Hy × R4 as they did in A × TR, especially in plants in the late stage of development, for there were 39 and 42 percent as many borers in Hy × R4 as in A × TR in the late and midseason stages, respectively, as compared with 45 percent in the early stage. In the 1936 experiment Hy × R4 contained about 46 percent as many mature borers as did A × TR in plants of the early stage, and this percentage increased to 86 (fig. 1) as the time from borer hatching to pollen shedding decreased from 14 days to 2 days.

TABLE 2.—*European corn borers of different ages infesting borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn in different stages of development at time of borer hatching, Toledo, Ohio, 1937*

Age of borers (days)	Borers per plant—(plants in early stage)			Borers per plant—(plants in midseason stage)			Borers per plant—(plants in late stage)		
	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
4 [1]	11.5	6.4	56	15.9	9.2	58	17.7	11.5	65
8	8.8	3.6		10.0	5.0		10.3	3.7	
13	5.9	2.6		6.3	4.0		9.8	3.0	
17	3.9	2.4		6.5	3.0		8.3	3.6	
22	3.0	2.1		6.6	2.4		7.1	2.6	
Mean: 15.0	5.40	2.68	50	7.35	3.60	49	8.88	3.23	36
26	3.9	2.4		4.5	2.6		6.8	2.8	
31	3.5	2.1		4.1	2.2		5.6	2.0	
36	3.1	2.1		4.3	1.6		5.2	2.4	
41	2.6	1.2		5.1	2.4		5.6	2.4	
Mean: 33.5	3.28	1.95	59	4.50	2.20	49	5.80	2.40	41
45	3.0	1.3		4.9	2.0		6.1	2.1	
50	3.0	1.6		3.7	1.6		5.1	2.1	
55	3.6	1.4		3.8	1.6		4.0	1.8	
Mean: 50.0	3.20	1.43	45	4.13	1.73	42	5.07	2.00	39

[1] Data for borers 4 days old not included in calculating means for the age group 8 to 22 days.

Table 2 also shows that the borer population in both hybrids decreased considerably with advancing borer age, most of the decrease in Hy × R4 occurring before the eighth day. The ratio of the number of borers in Hy × R4 to the number in A × TR, however, decreased little after the fourth day. At 4 days old from 56 to 65 percent as many borers were found in Hy × R4 as in A × TR, depending on the stage of the plants, and with borers averaging 50.0 days old the percentages ranged from 45 to 39, but it is doubtful whether these percentages differ significantly from those based on the two preceding age groups. Most of the differentiation in the borer population between the two hybrids therefore occurred before the borers were 4 days old.

More detailed data from the plants infested on July 8 give totals of 506, 587, and 343 young larvae in 24 plants of A × TR on the first, second, and fourth days after hatching. Samples of Hy × R4 contained 88, 55, and 49 percent as many larvae, respectively, showing

again that the differentiation in the number of borers in the two hybrids occurred within the first 4 days after hatching.

COMPARATIVE WEIGHTS OF BORERS AT DIFFERENT AGES

At various times during the first 57 days of larval life borers were dissected from the sample plants and weighed on the day of dissection. In the course of dissection many borers were accidentally cut and were not weighed. All the borers taken from the tassels of Hy × R4 and available for weighing totaled less than 100, and no comparisons were possible between these borers and borers from other locations. The samples of borers from the tassels of A × TR, however, were adequate, and the weight of borers of the same age averaged more than that of borers taken from other parts of the plant. When 7.4, 13.5, 19.1, and 24.6 days old, borers from parts of plants other than the tassels weighed 69.8, 82.4, 81.1, and 76.3 percent as much, respectively, as borers taken from the tassel buds or tassel stalks. The young borers up to 25 days old evidently found the tassels more nutritious than other parts of the plant.

Comparisons were made between the weights of borers taken from the entire plants of A × TR and Hy × R4. The growth curve of the borers from the 4 latest plantings of Hy × R4 did not differ essentially from that of the borers from the 4 earliest plantings. The borers from the 4 earliest plantings of A × TR weighed more than the borers from the 4 latest plantings, probably in part because 39 percent were from the tassels in the 4 earliest plantings as compared with 12 percent in the 4 latest plantings. Table 3 gives the number of borers and their mean weights from the 4 earliest and 4 latest plantings of A × TR and from these plantings combined in the case of Hy × R4. A sample of 200 newly hatched borers averaged 0.11 mg. per borer, but not until the borers were 12 days old were weighings of borers from all plantings made.

TABLE 3.—*Weights of European corn borer larvae of different ages taken from the entire plants of borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1937*

Mean age of borers (days)	A × TR				Hy × R4		Ratio of weight of borers in Hy × R4 to those in A × TR[1]	
	4 earliest plantings		4 latest plantings		Total of 4 earliest and 4 latest plantings			
	Borers	Mean weight	Borers	Mean weight	Borers	Mean weight	4 earliest plantings	4 latest plantings
	Number	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Percent*	*Percent*
13.6	260	7.0	148	5.2	136	5.6	80.0	107.7
18.4	230	10.8	60	8.2	144	7.0	64.8	85.4
23.1	191	24.5	76	17.2	116	15.9	64.9	92.4
27.8	167	42.9	102	33.2	105	29.6	69.0	89.2
32.5	161	71.7	59	51.4	94	36.3	50.6	70.6
37.2	143	93.9	65	88.3	108	51.8	55.2	58.7
41.7	179	105.1	69	99.7	97	83.0	79.0	83.2
46.4	151	121.7	62	108.9	90	93.1	76.5	85.5
51.2	130	113.5	74	119.6	99	94.4	83.2	78.9
56.8	210	122.0	114	116.1	118	110.8	90.8	95.4

[1] Data for Hy × R4 are based upon the total of the 4 earliest plantings and the 4 latest plantings.

The ratios of weights of borers in Hy × R4 relative to those in A × TR decreased with advancing age until about 33 to 37 days

after hatching and then increased as the borers approached maturity. This relationship indicates that during the first half of the growth period the borers in A × TR grew faster than those in Hy × R4, but during the last half of the period they approached their full-fed weight

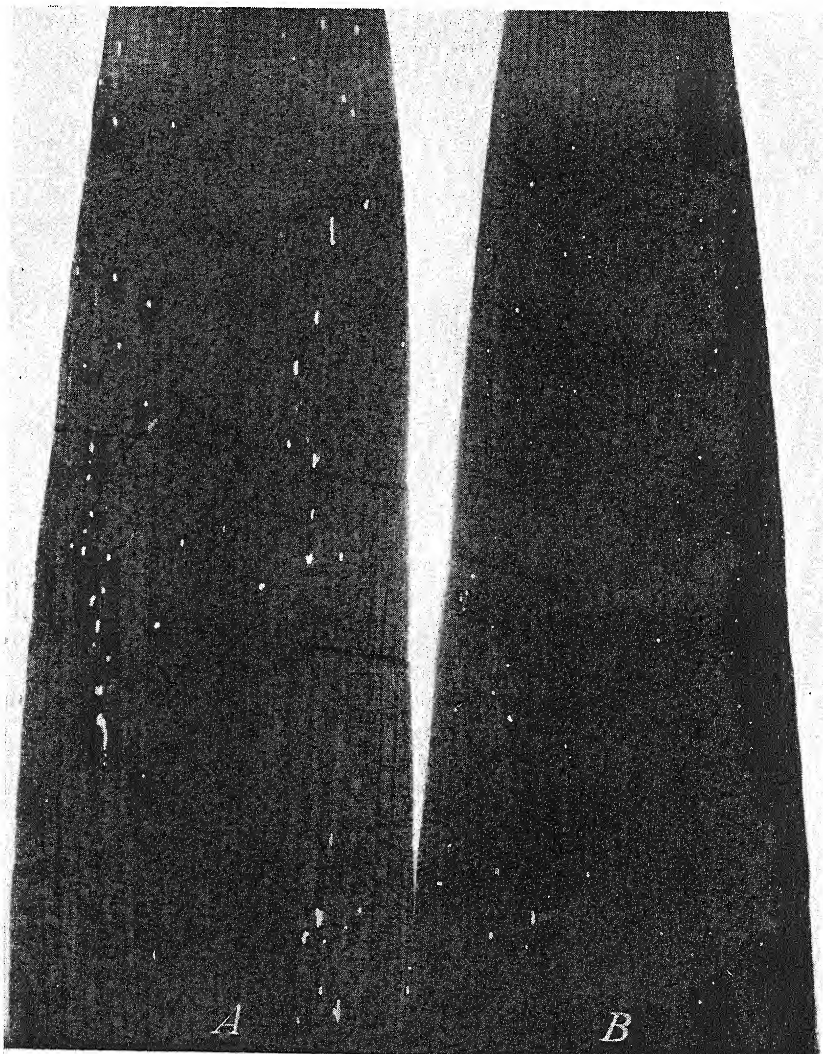

FIGURE 3.—Two types of feeding areas of the European corn borer on the leaf tissue of field corn: *A*, Borer-susceptible single-cross hybrid A × TR; *B*, borer-resistant Hy × R4.

relatively more slowly, whereas the borers in Hy × R4 gained enough more in weight during the last half of the growth period partly to overcome their under size during the first half. When 56.8 days old the borers in Hy × R4 weighed more than 90 percent as much as the borers in A × TR.

Averages of the ratios in table 3 show that from 18.4 to 46.4 days after hatching the borers in Hy × R4 weighed 65.7 percent as much as those in the first four plantings, and 80.7 percent as much as those in the last four plantings of A × TR. From the data in table 2 it may be seen that during the period 17 to 45 days after the hatching of a given number of eggs the survival on Hy × R4 averaged 48 percent of the survival on A × TR. Thus, since both the weight of the surviving borers and the percentage of survival were lower in Hy × R4 than in A × TR, it is evident that the former is a less suitable food for the borers.

In the preceding section it was shown that the earlier plantings provided better conditions for the survival of borers in both hybrids, especially in A × TR (table 2). A comparison of the weight ratios of 65.7 and 80.7 percent, coupled with the fact that the growth curves for larvae in the four earliest and four latest plantings of Hy × R4 were the same, indicates that the borers in A × TR gained more in growth over the borers in Hy × R4 in the earliest plantings than in the latest plantings. Hence, the borers in the earliest plantings of A × TR compared with borers in the latest plantings encountered better conditions for survival and also for a more rapid growth than did the borers in corresponding plantings of Hy × R4.

The feeding responses of newly hatched larvae on the leaf tissue of Hy × R4 also indicate that this hybrid is imperfectly adapted to them. The pin-point feeding areas on Hy × R4, as shown in figure 3, suggest that the young larvae made numerous attempts to find suitable food, whereas the narrow, elongated feeding areas on A × TR suggest that the larvae found suitable food on the first trial and remained to complete feeding. Bottger's [6] observations further suggest that the fine-textured excrement, greatly changed in color, from borers feeding on internodes of A × TR indicates a more nearly complete digestion than does the coarse-tectured excrement, retaining the original color of the food, from borers in Hy × R4.

LOCATION OF BORERS IN THE PLANTS

The basic data summarized for table 2 were combined further to learn the feeding locations in the two hybrids at different ages of borers. Table 4 gives the percentages of the total number of borers feeding in different locations for four age groups and for four groups of plants differing in stage of development. Since it has been shown in table 2 that about the same percentage of borers died with advancing age in Hy × R4 as in A × TR beyond the fourth day after hatching, a direct comparison may be made between the percentages of the borers in the different locations in the two hybrids.

The first marked difference observed in the location of the borers was in their distribution between the tassels and whorls up to the time they were 13 days old. The larger percentage found in the tassels of some plants was offset by a nearly equal larger percentage in the whorls of other plants. This difference in location occurred both between plants of A × TR and Hy × R4 in nearly the same stage of development at time of borer hatch but with a delayed eclosion of the tassels in Hy × R4 and between plants of A × TR in a relatively

[6] BOTTGER, G. T. PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER Jour. Agr. Res. 60: 249–258. 1940.

late stage and plants in an early stage with undeveloped tassels. When borers 4 to 13 days old hatched from 14 to 20 days before pollen shedding, averages of 39.2 and 25.1 percent were in the tassels and whorls of A × TR, respectively, as compared with 7.4 and 51.2 percent in Hy × R4. The 31.8 larger percentage of borers in the tassels of A × TR does not differ greatly from the 26.1 larger percentage in the whorls of Hy × R4. When borers of the same age hatched from 21 to 32 days before pollen shedding, averages of 6.5 and 64.4 percent were in the tassels and whorls, respectively, of A × TR. The 32.7 larger percentage in the tassels of the relatively more mature plants of A × TR does not differ greatly from the 39.3 larger percentage of borers in the whorls of the less mature plants of A × TR.

TABLE 4.—*Percentage of the total number of European corn borers of different ages located in the parts of plants of borer-resistant (Hy × R4) as compared with borer-susceptible (A × TR) single-cross hybrid field corn when the borers hatched 14 to 32 days before the plants shed pollen, Toledo, Ohio, 1937*

Age of borers (days)	Interval between borer hatching and pollen shedding	Total borers in 72 plants		Borers found—																	
				Within tassel buds or stalks		On leaf										Within ear buds or ears		Within internodes of stalk	
						Within whorl		Under ligules		Within midrib		On surface		Behind sheaths					
		Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR
	Days	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
4-13	14-17	492	1,068	12.0	53.8	44.0	13.4	28.1	19.0	4.3	1.8	2.5	1.3	7.2	6.8	1.9	1.7	0	2.2
	18-20	409	612	2.7	24.6	58.3	36.7	23.5	23.6	1.9	3.8	3.0	2.8	10.1	7.5	.5	.2	0	.8
	21-24	358	920	1.2	11.9	52.1	55.3	25.5	12.9	4.2	1.9	6.5	1.4	10.2	16.6	.3	0	0	0
	28-32	289	475	0	1.1	59.9	73.4	13.1	14.9	6.5	1.5	12.8	2.6	6.9	6.5	.8	0	0	0
	Mean	----	-----	4.0	22.9	53.6	44.7	22.5	17.6	4.2	2.3	6.2	2.0	8.6	9.3	.9	.5	0	.7
17-26	14-17	266	635	13.5	19.9	1.3	.5	35.8	27.7	9.5	3.1	2.8	.8	13.8	14.6	9.7	17.7	13.6	15.7
	18-20	150	352	14.2	24.6	6.3	1.4	44.1	33.7	10.7	5.1	2.5	2.3	9.4	9.8	2.4	10.1	10.4	13.0
	21-24	223	436	11.0	23.7	9.3	2.1	40.6	30.9	15.9	10.3	1.4	2.1	10.0	13.5	.9	3.1	10.9	14.3
	28-32	127	185	4.8	10.8	16.2	16.1	42.9	37.1	16.7	9.3	2.9	1.5	3.3	8.6	3.7	2.3	9.5	14.3
	Mean	----	-----	10.9	19.8	8.3	5.0	40.8	32.4	13.2	6.9	2.4	1.7	9.1	11.6	4.2	8.3	11.1	14.3
31-41	14-17	188	485	5.6	5.8	----	----	11.2	4.0	1.9	.2	1.1	0	13.1	8.0	26.4	30.9	40.7	51.1
	18-20	119	246	7.9	11.2	----	----	13.7	7.8	1.5	3.4	1.5	.5	19.2	10.6	14.8	23.9	41.4	42.6
	21-24	176	349	6.9	10.8	----	----	17.6	10.8	7.9	.9	1.3	.5	19.0	11.1	13.6	20.8	33.7	45.1
	28-32	103	159	10.7	10.7	----	----	16.5	10.1	4.4	1.3	1.7	1.2	12.1	10.0	5.1	11.9	49.5	54.8
	Mean	----	-----	7.8	9.6	----	----	14.8	8.2	3.9	1.5	1.4	.5	15.8	9.9	15.0	21.9	41.3	48.4
45-55	14-17	156	424	1.9	1.9	----	----	2.0	0	0	0	.6	.2	18.8	13.3	20.3	17.5	56.4	67.1
	18-20	122	266	1.6	1.8	----	----	.7	.3	.7	.3	0	.8	25.6	16.8	17.4	11.8	54.0	68.2
	21-24	142	355	2.9	3.6	----	----	0	.6	0	0	1.5	.6	18.2	9.1	11.3	18.2	66.1	67.9
	28-32	74	142	2.6	2.9	----	----	4.4	.8	1.2	0	0	0	20.6	15.8	8.9	10.4	62.3	70.1
	Mean	----	-----	2.2	2.5	----	----	1.8	.4	.5	.1	.5	.4	20.8	13.8	14.5	14.5	59.7	68.3

It was shown previously that the differentiation in the number of borers found in the two hybrids occurred before the borers were 4 days old. It was indicated also that 50.5 and 5.1 percent of the borers 4 days old were in the tassels of A × TR and Hy × R4, respectively, 14 to 17 days before the plants shed pollen. In other samples of plants of the same plantings, but infested earlier when the tassels were not available, during the first 4 days of larval life 662, or 70 percent, of 943 borers were within the whorl of leaves of Hy × R4, and 1,107, or 77 percent, of 1,436 borers were within the whorl of leaves of A × TR. Therefore, most of the differentiation in the

numbers of borers surviving in Hy × R4 and A × TR occurred in this part of the plant.

The percentage of borers of all ages in the tassels and the total percentage in the ears and internodes were larger in A × TR, but the total percentage in the whorls, ligules, and midribs was larger in Hy × R4. These differences are best shown in table 5, where the data from table 4 have been consolidated for two stages of the plants and four age groups of borers.

TABLE 5.—*Location of European corn borers of different ages in plants of borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1937. Consolidation of data in table 4*

Days between borer hatching and pollen shedding	Age of borers	Borers found— In tassels		In whorls, ligules, and midribs of leaves		In ear buds or ears and internodes of stalk		On leaf surface and behind leaf sheaths	
		Hy×R4	A×TR	Hy×R4	A×TR	Hy×R4	A×TR	Hy×R4	A×TR
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
14–20	4–13	7.4	39.2	80.0	49.2	1.2	2.5	11.4	9.2
	17–26	13.9	22.3	53.9	35.8	18.0	28.3	14.3	13.8
	31–41	6.8	8.5	14.2	7.7	61.7	74.3	17.5	9.6
	45–55	1.8	1.9	1.7	.3	74.0	82.3	22.5	15.5
21–32	4–13	.6	6.5	80.7	80.0	.6	0	18.2	13.6
	17–26	7.9	17.3	70.8	52.9	12.5	17.0	8.8	12.9
	31–41	8.8	10.8	23.2	11.6	51.0	66.3	17.0	11.4
	45–55	2.8	3.3	2.8	.7	74.3	83.3	20.1	12.8

For all age groups except one there was little difference between groups in the percentage of the borers in the tassels. For the borers in the first age group in plants in the later stage of development the percentage in the tassels of Hy × R4 was 31.8 less than in those of A × TR. Borers of the next age group showed a decrease in the percentage in the tassels of A × TR and an increase in those of Hy × R4. The delay in the eclosion of the tassels of Hy × R4 and the resulting greater inaccessibility of the tassel buds to the young larvae is the only known reason for the smaller percentages of borers in the tassels of that hybrid.

For the borers in the first age group in plants in the later stage of development there were 49.2 percent in the whorls, ligules, and midribs of A × TR as compared with 80.0 percent in those of Hy × R4. The difference, which was almost equal to the difference in the percentages in the tassels but reversed, showed the effect of the delayed tassel eclosion of Hy × R4 in keeping the young larvae from entering the tassels.

In plants in the early stage of development the two hybrids showed nearly equal total percentages of borers of the earliest age group in the whorls, ligules, and midribs, and most of the remaining borers were on the surface of leaves and behind leaf sheaths. At this stage the tassels in both hybrids were mostly undeveloped and only relatively few borers became established in them.

As the borers grew older they left the whorls, ligules, and midribs to go into the ear buds, or ears, and internodes. Some of the borers in the tassel buds entered the tassel stalks, and many went to other locations, finding eventual establishment in the ears and internodes.

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For all age groups, when more than 1 percent of the borers is considered, from about 5 to 15 percent more borers were in the ears and internodes of A × TR than of Hy × R4, an indication that they went to these locations earlier in A × TR. Consequently there were larger percentages in the whorl, ligules, and midribs of Hy × R4. The larger percentages of borers in the ears and internodes of A × TR were possibly associated with the larger size of the borers in this hybrid, the larger borers migrating to these parts before they did in Hy × R4.

For borers up to 26 days old the differences between the two hybrids in the percentages found on the surface of leaves and behind leaf sheaths are of minor consideration. The average for these borers in both plant-development groups was 13.2 percent in the case of Hy × R4 and 12.3 percent in the case of A × TR (table 5). For borers 31 to 55 days old on an average less than 1.5 per cent of the borers were on leaf surfaces in either hybrid (table 4). However, on an average 18.3 percent were behind the leaf sheaths in Hy × R4 as compared with 11.9 percent in A × TR.

The larger percentage behind the leaf sheaths of Hy × R4 was, again, probably a manifestation of the smaller size of the borers in that hybrid and of a resulting delay in entering the ears and internodes. The total percentage in the ears, internodes, and tassel stalks averaged 82.7 in A × TR as compared with 70.3 in Hy × R4. Hence, the smaller percentage in these locations in Hy × R4 indicates an equal larger percentage in other locations as compared with A × TR. Of the 12.4 larger percentage in other locations of Hy × R4, 5.4 percent were located at the ligules of the leaves and in the midribs and 6.4 percent were behind the leaf sheaths. Probably most of the borers behind the leaf sheaths and at the adjoining ligules were just about to bore into the internodes. Many of the borers were seen boring into the internodes when the plants were dissected.

To complete the data on the parts of the plants infested, the percentages of borers at 26 and 55 days old occurring in particular internodes were determined. Between these ages the relationship between percentages of borers and ages of borers in the internodes was found to be linear. A few borers were located in the thirteenth internode of both hybrids, or the internode just below the tassel. In the twelfth to ninth internodes, called the top third of the internodes, as the age of the borers increased 29 days, the 33 percent decreased to 14 in A × TR and 35 percent decreased to 25 in Hy × R4. In the eighth to fifth internodes 42 percent increased to 54 in A × TR and 41 percent remained constant in Hy × R4. In the lowest third of the internodes, or the 4 internodes above the surface of the ground, 19 percent increased to 28 in A × TR and 8 percent increased to 29 in Hy × R4. These data show that when the borers were about half grown nearly the same percentage were in the top third of the internodes of both hybrids and a smaller percentage were in the lowest third of the internodes of the resistant hybrid. With increasing age the borers left the top third of the internodes to enter those lower down, but they did so less extensively in the resistant hybrid.

SUMMARY

The discovery that a much smaller number of European corn borers matured from a given number of eggs hatching on resistant than on

susceptible field corn suggested that delayed eclosion of the tassel might be an important factor in borer survival. A study was therefore made of the effect of the stage of plant development and the presence of tassels at time of borer hatching on two single-cross hybrids, borer-resistant Ill. Hy × R4 and borer-susceptible Ill. A × Ind. TR. The comparative numbers and weights of the larvae from hatching to maturity and their location in the plants after the differentiation in numbers occurred were also studied.

Most of the differentiation in numbers of borers occurred within the first few days after hatching; thereafter survival was relatively the same in both hybrids.

The borers developed at a slower rate in the resistant than in the susceptible hybrid. Feeding characteristics as well as weights of borers showed the resistant hybrid to be less suitable as food.

The greater availability of the tassel buds in the susceptible hybrid seems to contribute to more rapid growth of the borers, but general unsuitability of the resistant hybrid for food is evidently a major factor in survival and probably in slower growth.

Differences in borer survival between the resistant and susceptible hybrid became less as the plants were nearer the pollen-shedding stage at time of borer hatching, both when detasseled and normal plants were used.

In plants shedding pollen 14 to 20 days after borer hatch, a much larger percentage of borers less than 13 days old were in the tassels of the susceptible hybrid, but almost an equally larger percentage were in the whorls, ligules, and midribs of the resistant hybrid. Most of the differentiation in survival occurred in the whorl of leaves. Larger percentages of the borers 17 to 55 days old were in the ears and internodes of the susceptible than of the resistant hybrid. When the borers were about half grown, nearly the same percentage were in the top third of the internodes of both hybrids and a smaller percentage in the lowest third of the internodes of the resistant hybrid. With increasing age the borers left the top third of the internodes to enter the internodes lower down, but they did so less extensively in the resistant hybrid.

A PYTHIUM STALK ROT OF CORN [1]

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INTRODUCTION

A stalk rot, later shown to be caused by a species of *Pythium*, was first observed by M. T. Jenkins [2] during late July and early August 1940, on the Potomac River bottoms of the Arlington Experiment Farm, Arlington, Va., on plants of two inbred lines of dent corn (*Zea mays* L.). All 17 plants in a row of K167, an inbred line developed at the Kansas Agricultural Experiment Station, were infected and, when 15 to 20 inches high, had fallen over just above the ground. The top of each plant could easily be lifted from the crown and root. Adjacent rows of K168 showed no infection. A few days after the discovery of the disease a similar stalk rot was observed in the same nursery in six rows of C. I.[3] 6, an inbred line developed by the Division of Cereal Crops and Diseases at the Arlington Farm. These plants were 5 feet tall and had stalks 1 inch or more in diameter. The six rows alternated with other inbreds, but infection occurred only in C. I. 6. A few plants in each of the six rows fell over, owing to rotting of the first or second internode above the soil line. In most cases the tops remained green and turgid for a week or more. Both infections appeared after a heavy rain and a subsequent period of very high temperature and high humidity. The infection did not spread and when the temperature went down appeared to cease as suddenly as it had developed.

THE DISEASE

The pythium stalk rot of corn considered here develops rapidly during a few days of very high temperature and high humidity, becomes apparent when the plants fall over, and then, when the temperature falls, progresses no farther. The fungus destroys the stalk tissue for 1 or 2 inches at one of the lower internodes, causing large plants to fall over, and then, instead of progressing to new tissue, apparently ceases its activity. The roots are abundant and healthy. There is no stunting of the plants. They are healthy and grow vigorously, and the disease becomes apparent only when the stalks bend abruptly at one of the lower internodes and fall to the ground (fig. 1). The outer rind of the cornstalk, the epidermis, the lignified cells of the hypodermis, and the inner parenchyma cells are softened, disorganized, and destroyed, leaving only the separate browned strands of vascular bundles, which cannot support the plant (fig. 2).

[1] Received for publication April 2, 1942.

[2] Principal agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases. Formerly, accession numbers for the inbred lines developed in this Division were designated by the prefix "U. S." instead of "C. I." but have been changed recently to avoid confusion with the designation of U. S. hybrids.

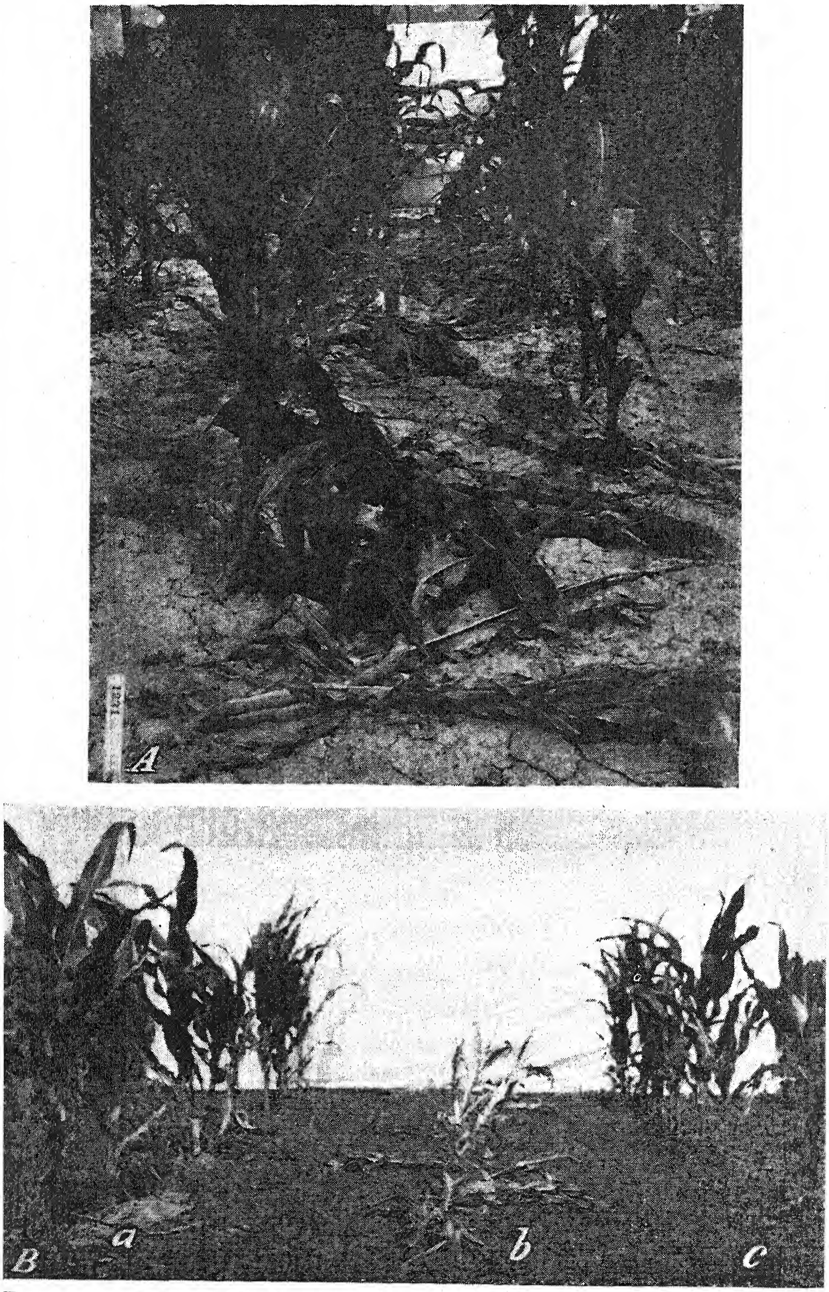

FIGURE 1.—Pythium stalk rot in inbred lines of yellow dent corn. Natural infection in the field at the Arlington Experiment Farm, Arlington, Va. Photographed by M. T. Jenkins. *A*, Row 1,231, C. I. 6. Photographed August 8, 1940. *B*, Inbred lines: *a*, K168; *b*, K167; *c*, K168. Photographed July 29, 1940.

In the lignified tissue of the older plants, the rotting usually is confined to and may involve not more than 2 inches of one internode. The vascular bundles were observed to continue to function in some of

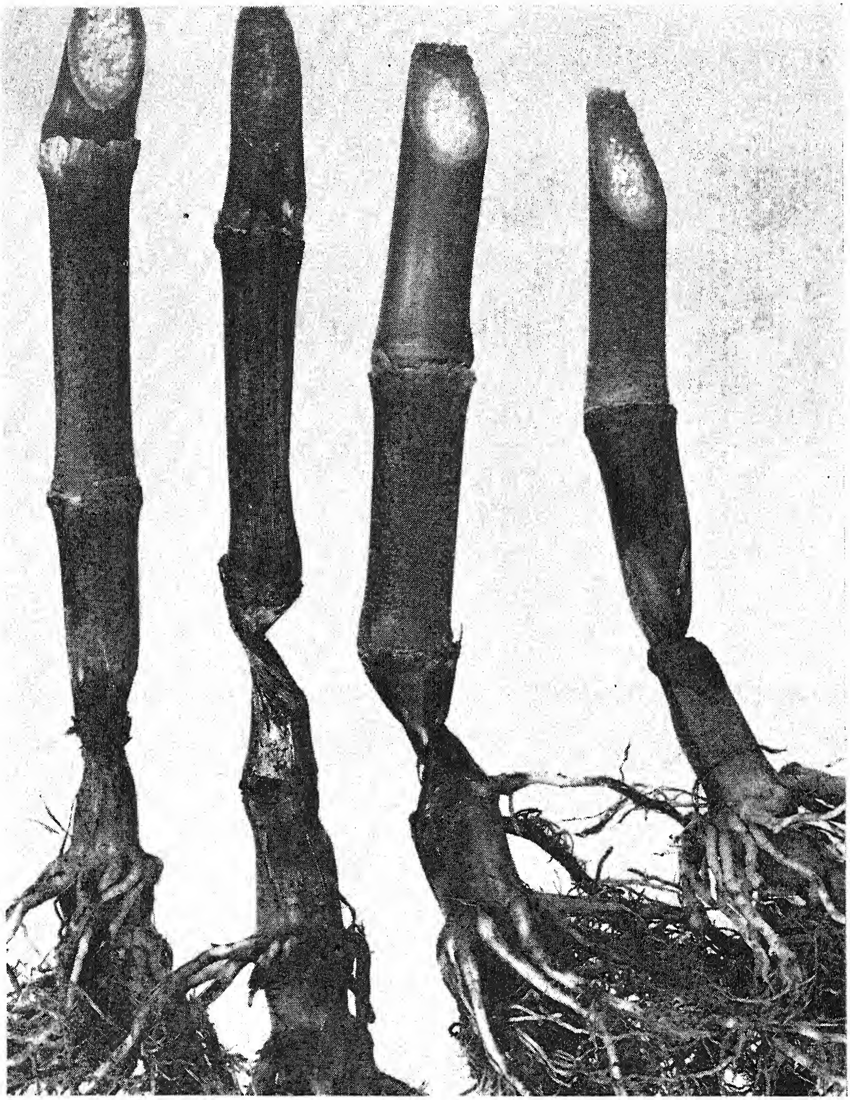

FIGURE 2.—Natural infection with pythium stalk rot in plants of inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Photographed August 6, 1940.

the large plants of inbred C. I. 6, so that the plants lying on the ground remained green and turgid for several days (fig. 1, *A*). Longitudinal sections of infected internodes showed water-soaked, brown tissue at the margins of infected areas and a sharp line of demarcation

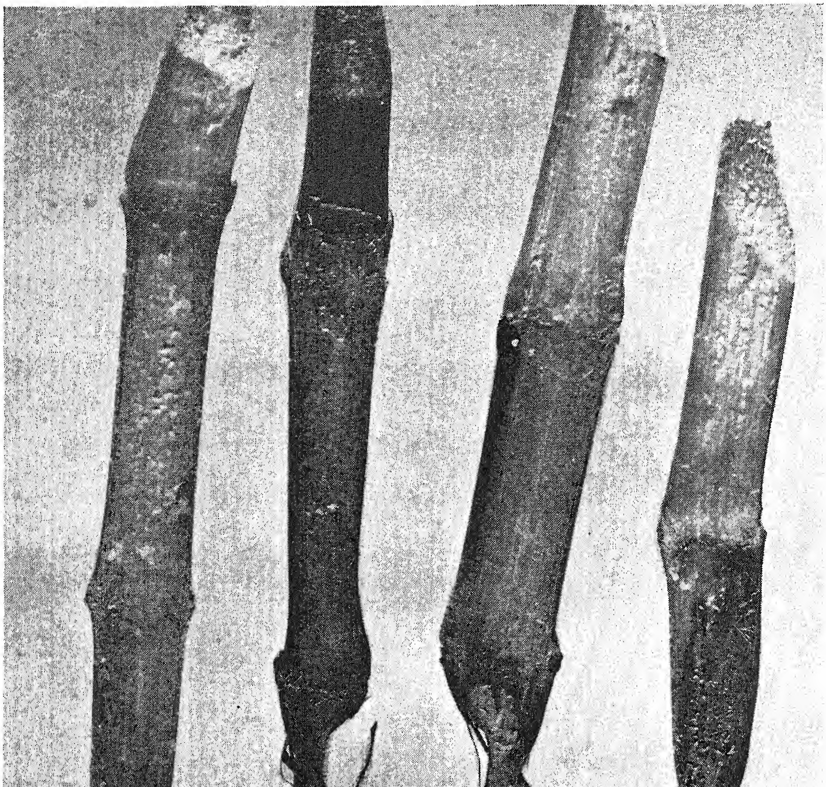

FIGURE 3.—Longitudinal sections showing natural infection with pythium stalk rot in plants of inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Photographed August 6, 1940.

between the dark-brown, water-soaked tissue and healthy tissue (fig. 3). Along this line there was often a purplish or lavender tinge.

At the Arlington Experiment Farm the infection was most severe in the tender tissue of the young plants of inbred K167, which were less than 2 feet tall, and developed first just above the ground level. It extended up the stalk through two to three internodes, or to the growing point, where infected tissue was much less clearly separated from the healthy. There was no putrid odor, as with bacterial infection, but a peculiar, characteristic odor, which has been described as "marshy."

A similar stalk rot was observed early in August 1941, in a small field of Hybrid Extra Early Yellow Dent corn growing on a farm west of Petersburg, Dinwiddie County, Va. The infection was scattered through the field but was more abundant on one side, where 20 to 25 percent of the stalks had lodged. The stalks were 1½ inches in diameter, and the plants had grown vigorously and normally with no signs of disease until they were found lying on the ground. Local rotting of internodes above the brace roots, like that in the plants of inbred C. I. 6, had caused them to lodge (figs. 4 and 5).

The field was visited about 3 weeks after the infection was first reported. Some plants had died, but in others the leaves and stalks above the rotted internodes were green and turgid, roots had developed at nodes just above the rotted internodes (figs. 5 and 6), and the growing tops had turned upward, developed tassels, and in one instance an ear (fig. 4). What was left of the rotted internodes was brown and dry. There was no evidence of bacterial infection and no odor of decay. In the interior of the stalks the rotted and healthy tissues were distinctly separated. The roots were abundant and healthy.

The development of this stalk rot in Dinwiddie County, Va., in 1941, followed a period of high temperatures, as was the case in 1940, at the Arlington Experiment Farm. The temperature and rainfall data for the two localities and for the period coinciding with or immediately preceding the observations of the disease are given in table 1.

TABLE 1.—*Temperature and rainfall data for the Arlington Experiment Farm and Richmond,*[1] *Va., for the periods approximately coinciding with the appearance of the stalk rot disease of corn*

Date	Arlington Experiment Farm, 1940		Richmond, Va., 1941		Date	Arlington Experiment Farm, 1940		Richmond, Va., 1941	
	Maximum temperature	Rainfall	Maximum temperature	Rainfall		Maximum temperature	Rainfall	Maximum temperature	Rainfall
	° F.	*Inches*	*° F.*	*Inches*		*° F.*	*Inches*	*° F.*	*Inches*
July 15	84	0	80	0	July 24	92	3.20	86	0
16	86	0	84	0	25	96	0	89	0
17	87	0	87	.39	26	100	0	98	.48
18	93	0	88	.32	27	100	0	96	0
19	97	0	89	.62	28	102	0	98	0
20	98	0	85	.01	29	95	.05	90	0
21	99	0	84	0	30	98	.01	98	0
22	99	0	83	0	31	95	0	97	0
23	92	0	84	[2] T					

[1] The disease occurred about 5 miles west of Petersburg, Va., and 20 miles south of Richmond. Weather records for Petersburg were not available.
[2] T=trace.

THE CAUSAL ORGANISM

IDENTITY

Specimens of stalk rot from the Arlington Experiment Farm, held in the culture room in glass dishes during a hot week end, developed a mat of cottony white mycelium. Transfers were made from this mycelium, and isolations also were made from pieces of rotted tissue

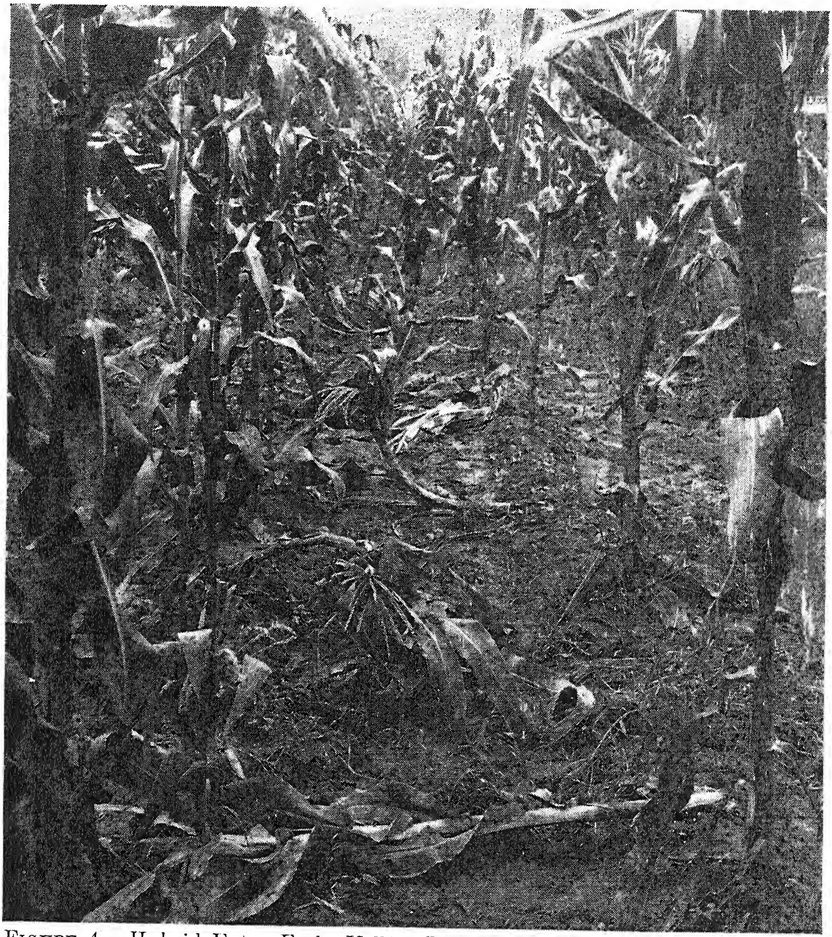

FIGURE 4.—Hybrid Extra Early Yellow Dent corn near Petersburg, Dinwiddie County, Va., where 20 to 25 percent of the stalks had rotted just above the ground and fallen over early in August. Photographed September 3, 1941.

washed in sterile water, held overnight in water in the refrigerator, and plated on water agar. Several isolations were made from both K167 (culture 40–F) and C. I. 6 (culture 40–G). Pure cultures showed a typical nonseptate mycelium, which when grown on corn meal-carrot agar developed an abundance of oogonia and antheridia. Pure cultures of the fungus were identified by Charles Drechsler [4] as *Pythium butleri* Subr. The history of this species has been reviewed

[4] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

by Drechsler[5] under the name of *P. aphanidermatum*. In 1934 he[6] separated *P. aphanidermatum* into two species on the basis

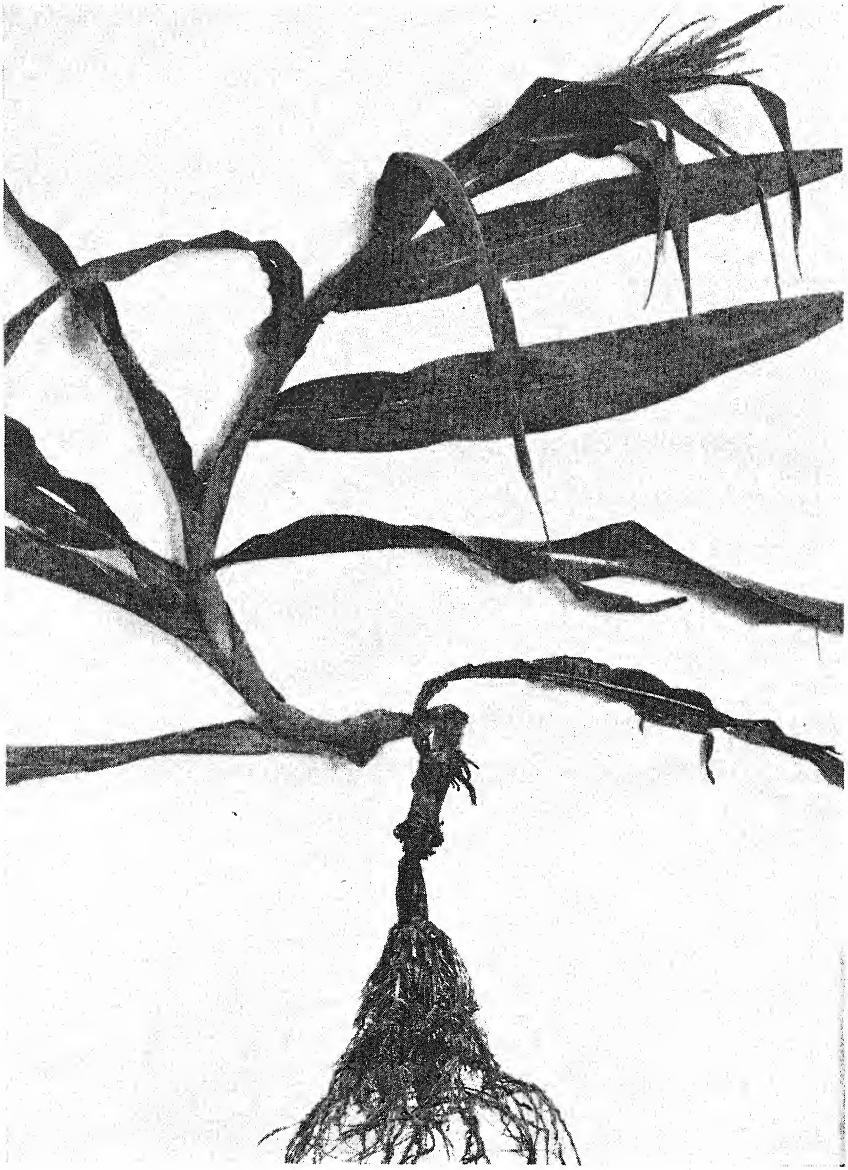

FIGURE 5.—Stalk rot and root formation at nodes above rotted tissue on Hybrid Extra Early Yellow Dent corn, near Petersburg, Va. Photographed September 4, 1941.

of size of fruiting bodies. Zoosporangia, zoospores, and sexual structures are larger in *P. butleri* than in *P. aphanidermatum*.

[5] DRECHSLER, C. THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM. Jour. Agr. Res. 30: 1035–1042, illus. 1925.

[6] DRECHSLER, C. PYTHIUM BUTLERI AND P. APHANIDERMATUM. (Abstract) Phytopathology 24: 7. 1934.

The host range and distribution of the two species are still somewhat confused. Although no extensive isolations have been made to determine the distribution of *P. butleri*, it is, according to Drechsler, a widely distributed fungus in warm climates, living near the surface of the soil and destructive to a variety of hosts. He reports [7] (p. 393) that during or immediately following periods of excessively hot moist weather this fungus may become especially destructive.

Repeated attempts were made to isolate an organism from the rotted stalks collected near Petersburg, Va., that would reproduce the disease. *Pythium*, however, was not isolated and none of the other fungi isolated produced infection. *Pythium butleri* used in the same inoculation test produced typical stalk rot in the greenhouse at Arlington Experiment Farm. The known difficulties in isolating *Pythium* in other cases suggest that the failure to secure *Pythium* from the Petersburg corn was due to lack of fresh material and actively developing infections. The infection in the field near Petersburg, therefore, probably was due to the same fungus that caused the stalk rot in the nursery at the Arlington Experiment Farm.

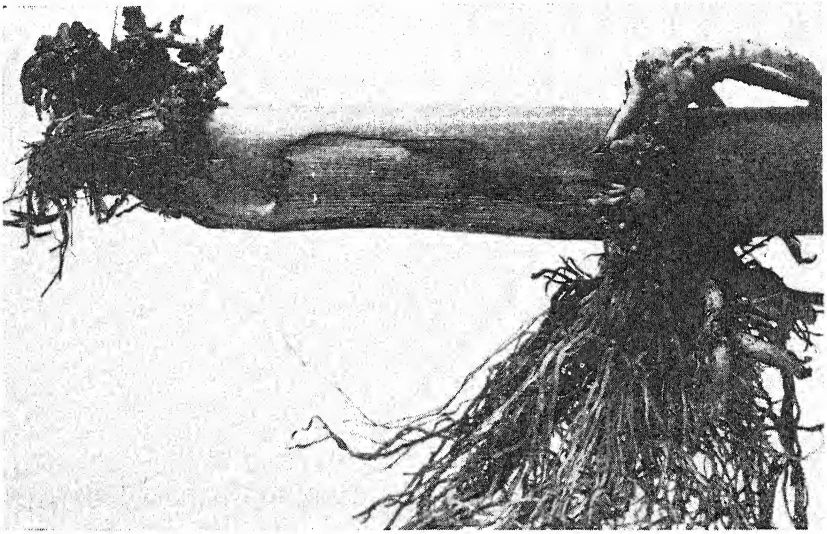

FIGURE 6.—Stalk rot, and root formation at nodes above rotted tissue, on Hybrid Extra Early Yellow Dent corn, near Petersburg, Va. Photographed September 4, 1941.

PATHOGENICITY

Cultures isolated from rotted stalks of two inbred lines of yellow dent corn, K167 and C. I. 6, were tested for pathogenicity in the field and in the greenhouse at the Arlington Experiment Farm.

FIELD INOCULATIONS

Healthy plants of inbred C. I. 6, in the rows where the infection had developed, were used for inoculation with isolations from plants of K167 and C. I. 6 on August 12, 1940. The surface of the stalk

[7] DRECHSLER, C. SEVERAL SPECIES OF PYTHIUM CAUSING BLOSSOM-END ROT OF WATERMELONS. Phytopathology 29: 391-422, illus. 1939.

was washed with alcohol and mercuric chloride, an incision made, and small pieces of agar culture inserted. Three plants were inoculated with each culture. Control plants were injured in the same way but no cultures were introduced.

Maximum temperatures following inoculation were too low to be favorable for rapid development of the disease. Results of this inoculation are given in table 2, and the temperature and rainfall

Table 2.—*Results of inoculations in the field, August 12, 1940, of large plants of inbred C. I. 6, yellow dent corn, with cultures isolated July 30 and Aug. 3, 1940, from stalk rot lesions on K167 and C. I. 6*

Culture No.	Plants—			
	Inoculated	Fallen over		With partial stalk rot
		Aug. 16	Sept. 11	
	Number	*Number*	*Number*	*Number*
40-F-1a [1]	3	0	0	3
40-F-2a [1]	3	0	0	0
40-F-3 [1]	3	0	0	3
40-G-3a [2]	3	0	0	0
40-G-4c [2]	3	1	1	3
40-G-5c [2]	3	0	0	0
40-G-5b [2]	3	0	0	3
Controls	3	0	0	0

[1] Isolated from inbred K167.
[2] Isolated from inbred C. I. 6.

records for the period from August 12 to September 22, 1940, at the Arlington Experiment Farm, are given in figure 7.

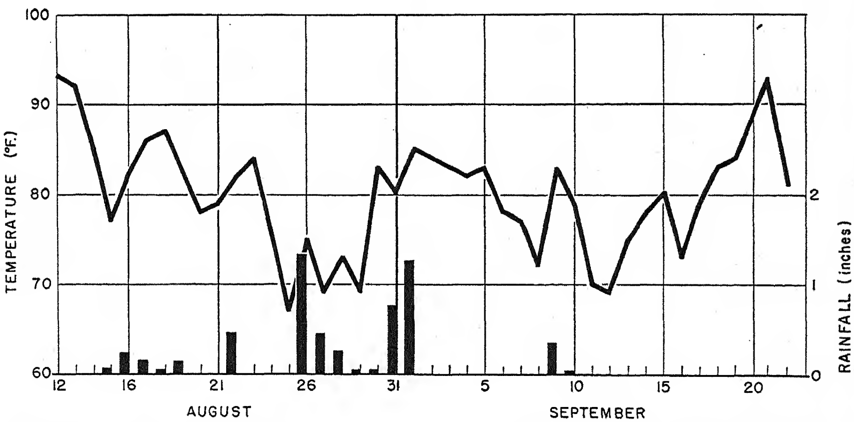

Figure 7.—Daily maximum temperatures and rainfall at the Arlington Experiment Farm from August 12 to September 22, 1940.

The results of an inoculation with culture 40-G-4c, which caused rotting of the inoculated internodes so that the plant fell over 4 days after inoculation, are shown in figure 8, *A*. A longitudinal section of this plant (fig. 8, *B*) shows the results of inoculations in two internodes. The infection in the lower internode progressed less rapidly than that in the upper. Some of the inoculated plants were not

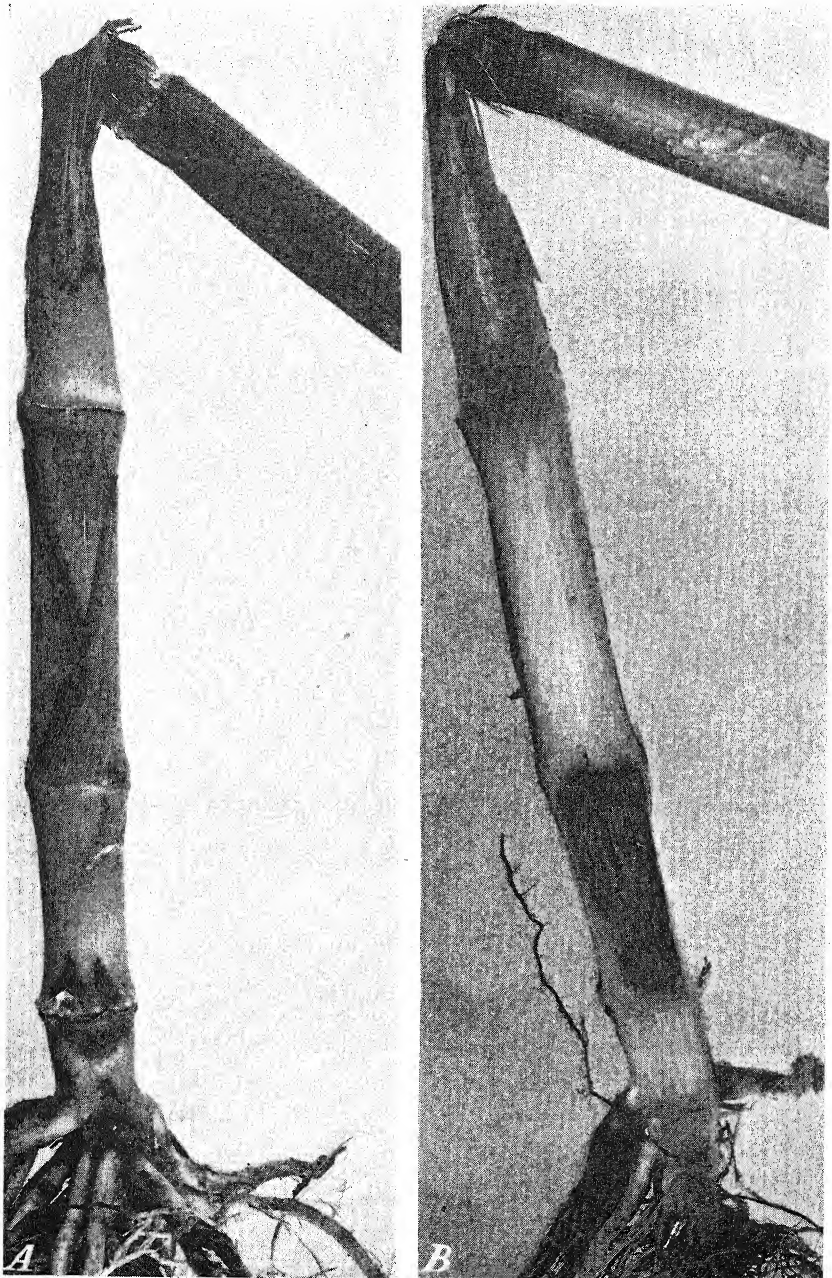

FIGURE 8.—Pythium stalk rot of C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Plant inoculated August 12, 1940, with *Pythium butleri*, culture 40–G–4c; fallen over August 16, 1940; photographed August 24, 1940. *A*, External view; *B*, longitudinal section.

sufficiently rotted to fall over. Longitudinal sections of representative stalks of these plants are shown in figure 9. The control plant showed some discoloration around the wound (fig. 9, *A*). All four inoculated stalks showed distinct rotting of the parenchyma tissue, particularly the stalk in figure 9, *B*, which was inoculated with culture

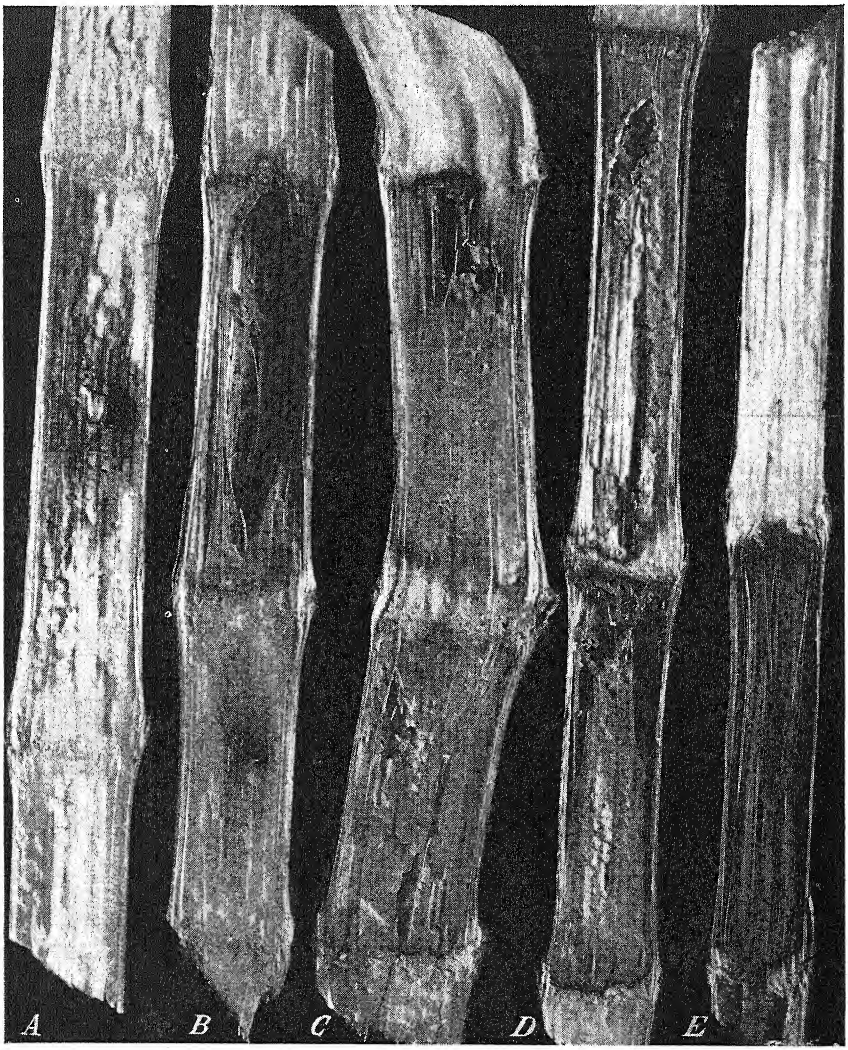

FIGURE 9.—Pythium stalk rot on inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Inoculated August 12, 1940, with cultures of *Pythium butleri* isolated from stalk rot; photographed September 9, 1940. *A*, Wounded, uninoculated; *B–E*, inoculated with cultures 40–G–4c, 40–F–1a_1, 40–G–5b, and 40–F–3, respectively.

40–G–4c isolated from C. I. 6, and that in figure 9, *E*, which was inoculated with culture 40–F–3 isolated from K167. The latter stalk easily yielded to pressure, and there was little tissue left except the vascular bundles and part of the outer rind. All three plants

inoculated with culture 40–G–5b (fig. 9, *D*) showed typical water-soaked rotting and brown margins. Although the infection did not progress far enough for the plants to fall over, they were so weakened that the rind easily yielded to pressure. All three plants inoculated with culture 40–F–1a$_1$ (fig. 9, *C*) also showed typical rotting. There was no putrid odor from the infected tissue in any of these plants. *Pythium butleri* was reisolated from several of the rotted stalks.

Seed of inbred C. I. 6 was planted in the field at the Arlington Experiment Farm early in August 1940. On September 12, when the plants were about 12 inches high, inoculations were made with 13 isolations. Of six plants inoculated with each culture, two were uninjured and had the culture placed between leaf sheath and stalk, two had the stalk scratched with a needle and the culture placed between leaf sheath and stalk, and two had cultures inserted into a scalpel wound in the stalk. No infection resulted in any of these inoculated plants, apparently because environmental conditions were unfavorable. For over a week following this inoculation, daily maximum temperatures were below 90° F. and there was no rainfall.

Greenhouse Inoculations

Inoculations in the greenhouse were made (1) to determine whether *Pythium butleri* would produce a root rot of dent corn inbred lines similar to that caused by *P. arrhenomanes* as described previously,[8] (2) to obtain additional proof of the pathogenicity of the cultures of *P. butleri* isolated from the rotted stalks of dent corn described above, and (3) to test the relative susceptibility of inbred lines of dent corn to this species of *Pythium*.

In all greenhouse inoculation tests, plants were grown in sterilized white sand, to which iron as iron magnetite, 1 part in 100 by weight, was added before sterilization. The plants were grown in 6-inch pots, washed before each test for 5 minutes in 1:50 commercial formaldehyde solution, and watered with a nutrient solution, as described in a previous publication.[8] Greenhouse benches were sprayed with the formaldehyde solution. The seed was rinsed in alcohol and held for 10 minutes in a 1:1,000 mercuric chloride solution, rinsed, and germinated in Petri dishes on wet filter paper. After planting, the seedlings were allowed to grow for several days before inoculation or until they were well established.

The first test was begun November 30, 1940. In this test, the sand in 40 pots containing plants of inbred C. I. 6 was inoculated by placing centimeter squares of agar cultures near the edge of the pot, on opposite sides of the plant. Four cultures were used, 40–F–3 and 40–F–1a$_1$, isolated from inbred K167, and 40–G–5b and 40–G–4c, isolated from inbred C. I. 6. Ten pots were inoculated with each culture, and corresponding sterile agar was added to the sand in 10 control pots. Weekly measurements of height showed the same increase in inoculated and control plants. On December 16, when the sand was washed from the roots, there was no evidence of root rot. Daily maximum temperatures in the greenhouse during this period ranged from 78° to 104° F.

In May 1941, a second test for root rot was made by similar sand inoculations of 13 inbred lines of dent corn. All seedlings, after

[8] Elliott, C. relative susceptibility to pythium root rot of twelve dent corn inbreds. Jour. Agr. Res. 64: 711–723, illus. 1942.

being transferred from Petri dishes to sand April 26, had grown for 2 weeks on the same greenhouse bench. Daily maximum temperatures in the greenhouse at Arlington Experiment Farm during the experiment for May and June 1941 are given in figure 10.

The results agree with those of the previous test. The mean heights of the plants of all 13 inbreds on May 12, when the inoculations were made, were as follows: Uninoculated, 32.8 cm.; inoculated, 35.1 cm. At the end of the experiment the respective means were 111.2 and 118.2 cm. Rates of growth were not retarded by the inoculation, and when the sand was washed from the roots, more than 4 weeks

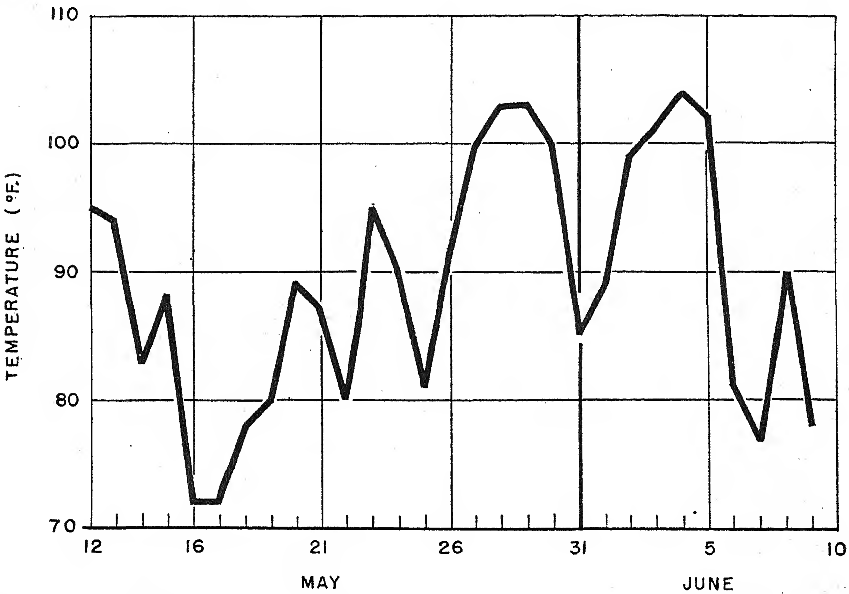

FIGURE 10.—Daily maximum temperatures in the greenhouse at the Arlington Experiment Farm during May and June 1941.

after inoculation, there was no evidence of root rot in any of the inbred lines.

Another test for root rot was made during the summer of 1941. On June 27, 13 inbred lines were grown in sand cultures and inoculated by placing cultures of 40–G–4c in the sand on opposite sides of each plant. Four pots of each were inoculated, and two of each were used as controls. Plants in inoculated sand grew as well as those in uninoculated sand, and when the sand was washed from the roots, August 4, there was no evidence of root rot. Roots in both inoculated and control plants were abundant and not discolored.

Direct inoculations of corn plants in the greenhouse to test the pathogenicity of fungus isolations from stalk rot were begun December 7, 1940. Five plants were inoculated with each of four cultures by placing pieces of agar cultures inside the lower leaf sheath. So far as could be observed, the plants had not been wounded. The results of this inoculation are given in table 3. Daily maximum temperatures in the greenhouse at Arlington Experiment Farm during the experiment, December 7 to 17, were 82°, 98°, 94°, 93°,

90°, 96°, 104°, 90°, 94°, 100°, and 97° F., respectively. Four days after inoculation, three plants inoculated with culture 40-F-3 developed stalk rot near the point of inoculation and fell over. One plant inoculated with culture 40-F-1a_1 had fallen over. Five days after inoculation, all five plants inoculated with culture 40-F-3 and two inoculated with each of the other three cultures had rotted and fallen. None of the five controls became infected.

Figure 11 shows the results of the test just described. Apparently *Pythium butleri* is able to penetrate unwounded stalk tissue.

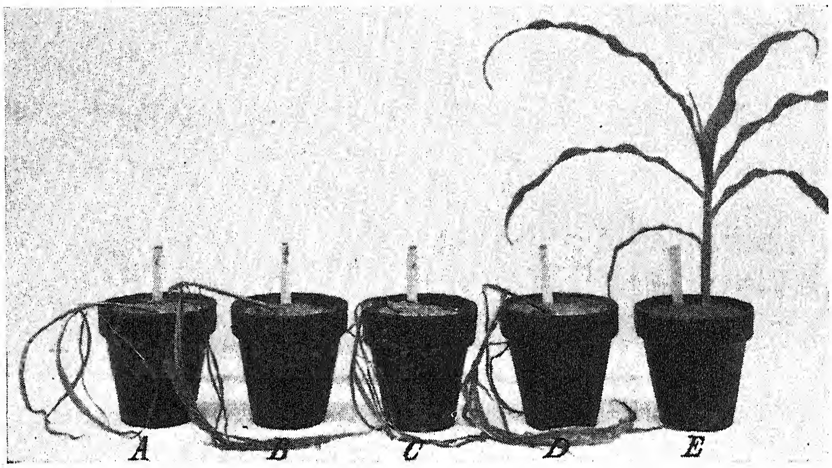

FIGURE 11.—Stalk rot of inbred C. I. 6, yellow dent corn, grown in sand plus nutrient solution in the greenhouse at the Arlington Experiment Farm. Inoculated December 7, 1940, with *Pythium butleri*, cultures (*A*) 40-F-3, (*B*) 40-G-5b, (*C*) 40-F-1a_1, and (*D*) 40-G-4c, respectively, isolated from rotted stalks. *E*, Uninoculated control. Photographed December 12, 1940.

TABLE 3.—*Results of greenhouse inoculations of inbred C. I. 6, yellow dent corn, grown in sand plus nutrient solution, with cultures of Pythium butleri isolated from stalk rot at Arlington Experiment Farm*

[Inoculations made without wounding plants, December 7, 1940]

Culture No.	Plants— Inoculated	Plants— Fallen over— Dec. 11	Plants— Fallen over— Dec. 12
	Number	*Number*	*Number*
40-F-3	5	3	5
40-G-5b	5	0	2
40-F-1a_1	5	1	2
40-G-4c	5	0	2
Controls	5	0	0

On June 6, 1941, 13 inbred lines of dent corn were inoculated to test the pathogenicity of culture 40-G-4c and to obtain information on possible differences in susceptibility to attack by *Pythium butleri*. Five plants of each inbred were inoculated by inserting mycelium into the stalk 1 to 2 inches above the soil line. Similar incisions were

made in 5 control plants of each inbred. The results are given in figure 12, *A* and *B*, and in table 4.

Maximum greenhouse temperatures for June 6, 7, 8, and 9 were 98°, 96°, 97°, and 94° F., respectively, and the humidity was high. On June 9, 3 days after inoculation, all but 8 of the 65 inoculated

FIGURE 12.—Thirteen inbred lines of dent corn, grown in sand plus nutrient solution; planted April 26, 1941. *A*, Stalks wounded at base June 6, 1941, but not inoculated; photographed June 9, 1941. *B*, Plant inoculated June 6, 1941, by wounding and inserting *Pythium butleri* into lower internode of stalk; photographed June 9, 1941. (Plants at farther end of bench were not inoculated.)

plants had fallen over. The stalk tissue was water-soaked and rotted for 1 to 2 inches around the point of inoculation. The attendant who watered the plants over the week end said they were lying flat on the bench on Sunday, only 48 hours after inoculation. All the inoculated plants were cut lengthwise to determine the extent of

rotting. Only 1 inbred, Ill. Hy., showed any marked resistance. In this line, only 1 plant in 5 fell over and rotting around the point of inoculation was very limited. In Ky. 13, 3 out of 5 plants fell over, and likewise 4 out of 5 plants of Ill. R4 and Ia. L317. Five inbreds were very susceptible.

TABLE 4.—*Inbred lines of dent corn inoculated June 6, 1941, with Pythium butleri, culture 40–G–4c, to determine differences in susceptibility to stalk rot*

Inbred—	Plants— Inoculated	Plants— Fallen over June 9	Plants— With stalk rot	Reaction to inoculation
	Number	*Number*	*Percent*	
C. I. 6:				
Inoculated	5	5	100	Very susceptible.[1]
Control	5	0	0	
Ill. Hy.:				
Inoculated	5	1	20	Resistant.
Control	5	0	0	
C. I. 540:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	
C. I. 5:				
Inoculated	5	5	100	Moderately susceptible.
Control	5	0	0	
Ia. L289:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ia. Mc401:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ky. 13:				
Inoculated	5	3	60	Moderately susceptible.
Control	5	0	0	
C. I. 1:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ill. R4:				
Inoculated	5	4	80	Moderately susceptible.
Control	5	0	0	
C. I. 4–8:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	
Ia. L317:				
Inoculated	5	4	80	Moderately susceptible.
Control	5	0	0	
Ind. Tr.:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ia. B1 345:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	

[1] Very susceptible=rot spreading throughout the stalk and into the growing point; no sharp line of demarcation between rotted and healthy tissue. Susceptible=rot spreading beyond inoculated internode, but sharp line between infected and healthy tissue. Moderately susceptible=rotting mostly confined to inoculated internode. Resistant=very limited rotting around point of inoculation.

On July 29, 1941, a limited number of plants of the same 13 inbreds were inoculated with culture 40–G–4c, to test further differences in susceptibility and to test again the ability of the fungus to infect uninjured as well as injured stalk tissue. This experiment was discontinued August 4, 6 days after the inoculations were made. The results are given in table 5.

Daily maximum greenhouse temperatures during the period July 29 to August 4, 1941, were 91°, 96°, 100°, 100°, 100°, 102°, and 102° F., respectively. In spite of these temperatures, conditions apparently were less favorable for infection than in the previous test; infection developed less rapidly and differences between inbreds were somewhat more apparent. Ill. Hy. and Ky. 13 again were the most resistant inbreds. While none of the plants of C. I. 6 fell over, sections of

inoculated stalks showed definite internal rotting. Infection developed most rapidly in C. I. 540, C. I. 5, Ia. L289, and C. I. 1; C. I. 4–8 and Ind. Tr. were almost as susceptible. Infection again developed without wounding.

Table 5.—*Results of greenhouse inoculations of 13 inbred lines of dent corn with Pythium butleri, culture 40–G–4c, July 29, 1941, Arlington Experiment Farm*

Inbred and method of inoculation [1]	Plants— Inoculated	Rotted and fallen over— 3d day	4th day	6th day	Remarks
C. I. 6:					
Inoculated:	*Number*	*Number*	*Number*	*Number*	
Wounded	1	0	0	0	Internode water-soaked inside.
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ill. Hy.:					
Inoculated:					
Wounded	2	0	0	0	Brown rot ½ inch around inoculation.
Not wounded	3	0	0	0	
Control	1	0	0	0	
C. I. 540:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	1	1	1	
Control	2	0	0	0	
C. I. 5:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. L289:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	1	
Control	2	0	0	0	
Ia. Mc401:					
Inoculated:					
Wounded	1	0	0	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ky. 13:					
Inoculated:					
Wounded	1	0	0	0	Brown rot ½ inch around inoculation.
Not wounded	3	0	0	0	
Control	2	0	0	0	
C. I. 1:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ill. R4:					
Inoculated:					
Wounded	1	0	0	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
C. I. 4–8:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. L317:					
Inoculated:					
Wounded	1	0	0	0	Rotted 1 inch around inoculation.
Not wounded	3	0	0	1	
Control	2	0	0	0	
Ind. Tr.:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. B1 345:					
Inoculated:					
Wounded	1	0	0	0	
Not wounded	2	0	0	1	
Control	0	0	0	0	

[1] In each case the wounded inoculated plants had mycelium introduced into an incision in their stalks, and the unwounded ones had it placed between the stalk and lower leaf sheath.

On September 9, 1941, *Pythium butleri*, culture 40–G–4c, isolated originally from inbred C. I. 6, was used to inoculate cucumber (*Cucumis sativus* L.), and three kinds of squash (*Cucurbita pepo* L.), namely, crookneck, Patty Pan, and Vegetable Marrow, in evaporating dishes in the laboratory. The two latter had very young tender tissue; the two former, rather old and hard tissue. Incisions were made in each specimen and pieces of mycelium were introduced

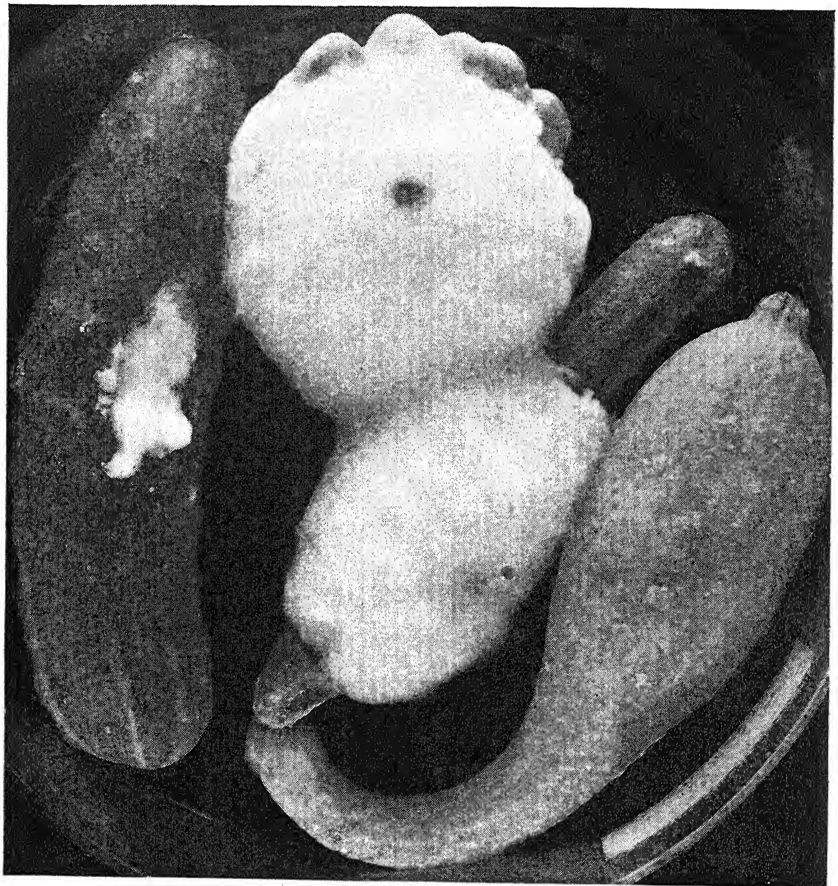

FIGURE 13.—*Pythium butleri*, culture 40–G–4c, on cucumber, Patty Pan squash crookneck squash, and Vegetable Marrow. Inoculated September 9, 1941; photographed September 11, 1941. September 15, 1941, all four were covered with an abundant mass of white mycelium.

Maximum temperatures in the laboratory on September 9 and 10 were 95° and 96° F. and the humidity was high. After 24 hours both Patty Pan squash and Vegetable Marrow showed water-soaked areas around the point of inoculation. Two days after inoculation these two squashes were almost covered with a cottony white mass of mycelium (fig. 13) similar to that described and illustrated by Drechsler [9] for this fungus. A white mycelium had broken out in several places for 2 inches around the inoculation on cucumber. Six days

[9] See footnote 5.

after inoculation, all four specimens were practically covered with an abundant growth of white mycelium.

CONTROL

Inasmuch as the disease developed only on certain corn inbreds in the nursery and not on inbreds in alternating rows, and as inoculations have shown that some inbreds are more resistant to infection than others, it seems that the best means of control is through the use of resistant strains.

Because of the exacting thermal requirement of *Pythium butleri*, it seems probable that this stalk rot may be of importance only in the southern parts of the Corn Belt.

SUMMARY

Stalk rot of two inbred lines (K167 and C. I. 6) of yellow dent corn (*Zea mays*) was observed in the field at the Arlington Experiment Farm in late July and early August 1940. The rotting in the larger plants was limited to one or more of the internodes above the brace roots. Infected plants fell over but remained green and turgid for several days. The infection became apparent after a period of hot humid weather. Successful inoculations with cultures isolated from this stalk rot were made during periods of high temperature and high humidity, but inoculations made when the temperature was lower produced little or no infection.

Pythium butleri Subr. was isolated from the rotted internodes and the disease was reproduced on inbred C. I. 6 in the field and in the greenhouse by inoculations with this fungus. Infections developed on both wounded and unwounded inoculated stalks.

Greenhouse inoculations on inbred C. I. 6 and on 12 other inbred lines of dent corn showed that some lines were more resistant than others. C. I. 5, Ia. L289, C. I. 1, and C. I. 540 were susceptible, and Ill. Hy. and Ky. 13 were resistant.

In greenhouse inoculations, *Pythium butleri* did not attack the corn roots.

Cultures of *Pythium butleri* isolated from rotted cornstalks rapidly rotted squashes and cucumber and covered them with a cottony mass of white mycelium, typical of this fungus.

What appeared to be the same disease occurred in a commercial field of hybrid corn near Petersburg, Va., August 1941.

FURTHER STUDIES ON INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA [1]

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INTRODUCTION

In an earlier paper, Thompson, Whitaker, and Kosar [3] reported on some genetic relationships between certain species of the 9-chromosome and of the 17-chromosome group of the genus *Lactuca*. Whitaker and Thompson [4] presented data on some cytological aspects of these same species. In the first of these papers mention was made of 2 of the 8-chromosome species, but no compatibility relationships were presented. The present paper shows some of the compatibility relationships found to exist between certain of the 8-chromosome species and species of the 9- and 17-chromosome groups. The studies on species relationships were undertaken for the purpose of isolating genetic factors for resistance to certain lettuce diseases, aster yellows in particular, and factors for hardiness and of determining the extent to which the species carrying such factors can be crossed with the cultivated form *Lactuca sativa*. This paper concludes certain phases of these studies and summarizes the contributions made to date.

MATERIALS AND METHODS

The original seed of the species used in the hybridization studies reported were obtained from the following sources:

Eight-chromosome species:

- *Lactuca bourgaei* (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.
- *L. marschallii* Stebbins, from Sweden, through G. L. Stebbins, Jr.
- *L. cretica* Desf., from Africa, through G. L. Stebbins, Jr.
- *L. capensis* var. *duruensis* De Wild., from Belgian Congo, through G. L. Stebbins, Jr.

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[1] Received for publication May 21, 1942.

[2] The writer is indebted to G. L. Stebbins, Jr., Department of Genetics, University of California, for seed and for identification of some of the species; to S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, for identification of some of the species and for verification of the botanical names; to the Division of Plant Exploration and Introduction for supplying seed of some of the foreign species; and to T. W. Whitaker, U. S. Department of Agriculture Horticultural Field Station, La Jolla, Calif., for the results presented in table 1 on the attempted crosses between the 8-chromosome species *Lactuca capensis* and 5 species representing the 8-, 9-, and 17-chromosome groups.

[3] Thompson, R. C., Whitaker, T. W., and Kosar, W. F. INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA. Jour. Agr. Res. 63:91–107, illus. 1941.

[4] Whitaker, T. W., and Thompson, R. C. CYTOLOGICAL STUDIES IN LACTUCA. Torrey Bot. Club Bul. 68:388-394, illus. 1941.

Journal of Agricultural Research, Washington, D. C.

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Nine-chromosome species:

- *L. tatarica* (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.
- *L. indica* L., from China, through Division of Plant Exploration and Introduction.
- *L. raddeana* Maxim., from Union of Soviet Socialist Republics, through Division of Plant Exploration and Introduction.
- *L. squarrosa* (Thunb.) Miq.,[5] from Asia, through Division of Plant Exploration and Introduction.
- *L. perennis* L., from Wayside Gardens, Mentor, Ohio.
- *L. serriola* L., collected in District of Columbia.
- *L. saligna* L., from Ohio, through G. L. Stebbins, Jr.
- *L. virosa* L., from Surrey, England, through Division of Plant Exploration and Introduction.
- *L. sativa* L., from private stocks.

Seventeen-chromosome species:

- *L. canadensis* L., collected in Maryland.
- *L. spicata* (Lam.) Hitchc., collected in Virginia.
- *L. floridana* (L.) Gaertn., collected in Maryland.
- *L. graminifolia* Michx., from South Carolina, through the late J. B. Norton.

All the parent and hybrid plants were grown in 10-inch clay pots under partly controlled conditions in a closely screened greenhouse at Beltsville, Md.

Except in the case of *Lactuca tatarica*, which was entirely self-sterile under the conditions prevailing in the greenhouse, flower heads of the maternal parents were depollinated with water. Pollen from the desired male parent was transferred by touching the pollen-laden stigmas and styles to the washed stigmas of the female flowers. Ten or more flower heads were pollinated in each attempted cross.

RESULTS

The results obtained from crosses attempted between the 8-chromosome species and species of the 8-, 9-, and 17-chromosome groups are presented in table 1.

TABLE 1.—*Summary of data on interspecific hybridization of some 8-chromosome species with certain 8-, 9-, and 17-chromosome species of Lactuca* [1]

Species and number of chromosomes	Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. bourgaei (8) ×:				
L. tatarica (9)	*O*	*T*		*S*
L. indica (9)	*O*	*O*		
L. squarrosa (9)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*N*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*N*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*N*	*T*		*S*
L. spicata (17)	*N*	*T*		*U*
L. graminifolia (17)	*N*	*T*		*S*
L. floridana (17)	*N*	*O*		
L. marschallii (8)	*T*	*T*	*F*	*F*
L. cretica (8)	*O*	*O*		

See footnotes at end of table.

[5] Called *Lactuca laciniata* in the paper cited in footnote 3. The writer has been informed by S. F. Blake and G. L. Stebbins, Jr., that the species formerly called *L. laciniata* should be classified as *L. squarrosa*.

TABLE 1.—*Summary of data on interspecific hybridization of some 8-chromosome species with certain 8-, 9-, and 17-chromosome species of Lactuca* [1]—Continued

Species and number of chromosomes	Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. marschallii (8) ×:				
L. indica (9)	*O*	*O*		
L. squarrosa (9)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*N*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*O*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*O*	*N*		
L. graminifolia (17)	*N*	*T*		*S*
L. floridana (17)	*N*	*T*		*S*
L. cretica (8) ×:				
L. tatarica (9)	*T*	*T*	*S*	*S*
L. indica (9)	*O*	*O*		
L. squarrosa (9)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*O*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*O*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*O*	*N*		
L. graminifolia (17)	*O*	*N*		
L. floridana (17)	*O*	*N*		
L. spicata (17)	*O*	*N*		
L. capensis (8) ×: [2]				
L. cretica (8)	*O*	*N*		
L. sativa (9)	*O*	*N*		
L. virosa (9)	*O*	*N*		
L. perennis (9)	*O*	*N*		
L. graminifolia (17)	*O*	*N*		

[1] Key to letter symbols:
F, the hybrids obtained were self-fertile.
N, the cross was not attempted.
O, the cross was attempted but failed.
S, hybrids obtained were self-sterile.
T, hybrid seedlings were obtained.
U, the fertility of this hybrid is not yet known.

[2] Data furnished by T. W. Whitaker.

In the investigations reported herein, 69 crosses were attempted between the 8-chromosome species and species with 8, 9, or 17 chromosomes; 25 of these were reciprocal crosses. Hybrids were obtained in only 8 of the 44 interspecific matings. All of these hybrids were self-sterile except those from the cross between the two 8-chromosome species, *Lactuca bourgaei* × *L. marschallii*, which were highly fertile, and the one from *L. spicata* × *L. bourgaei*, which had not produced flowers 9 months after germination. In 2 cases the hybrids obtained were from crosses between 8- and 9-chromosome species and in 5 cases from crosses between 8- and 17-chromosome species. Although in a few cases reciprocal hybrids were obtained, study was made only on plants from 1 group, as indicated by the order in which the species names are given in the discussion of each hybrid.

CROSSES BETWEEN EIGHT-CHROMOSOME SPECIES

Lactuca bourgaei (8) × *L. marschallii* (8) (fig. 1, *A*).—Reciprocal crosses between these two species were easily obtained, and fertile progenies resulted in both cases. The similarity of these two forms in many of their morphological characters combined with their breeding behavior indicates that they should be considered as varieties of the same species.

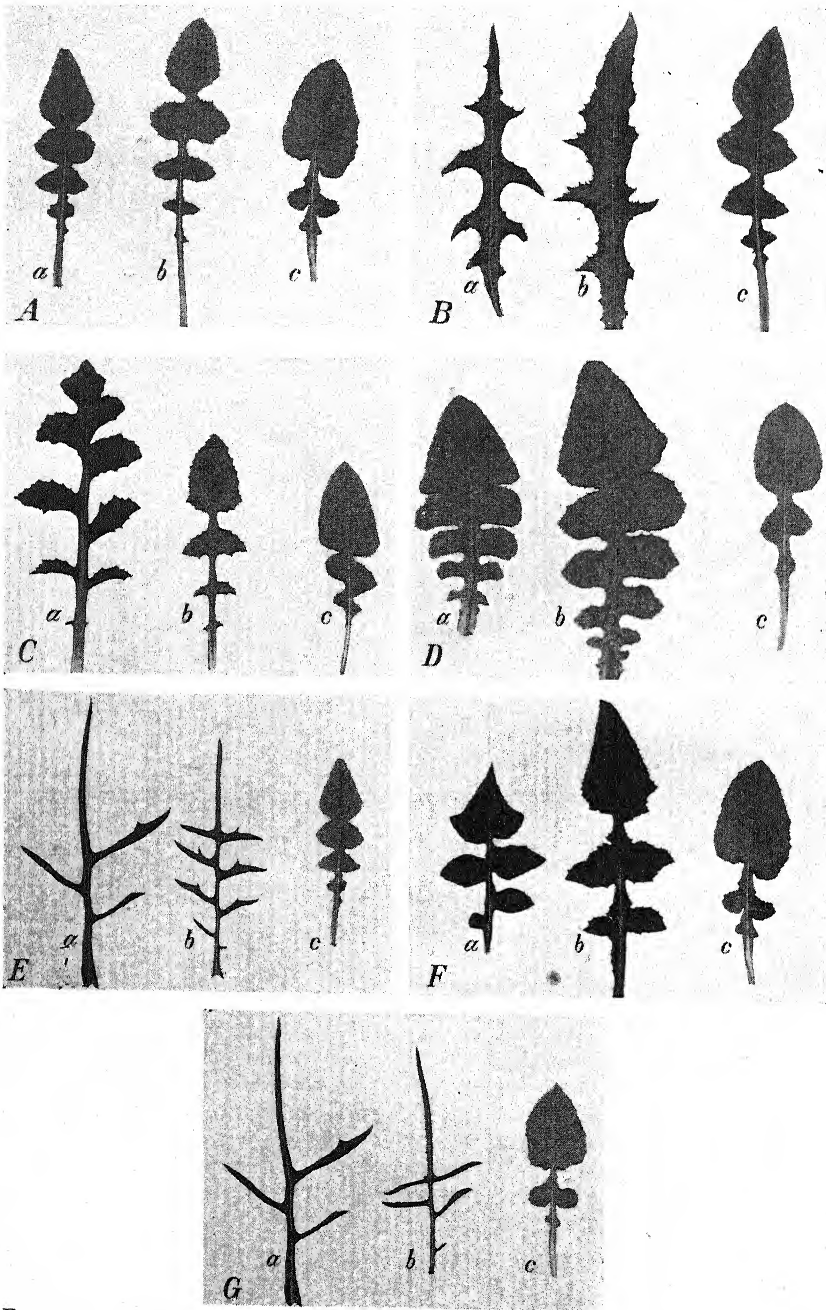

FIGURE 1.—Representative rosette leaves of various F_1 *Lactuca* hybrids and their parents. In each group *a* indicates the female parent, *b* the hybrid, and *c* the male parent. *A*, *L. bourgaei* × *L. marschallii*; *B*, *L. atarica* × *L. bourgaei*; *C*, *L. canadensis* × *L. bourgaei*; *D*, *L. spicata* × *L. bourgaei*; *E*, *L. graminifolia* × *L. bourgaei*; *F*, *L. floridana* × *L. marschallii*; *G*, *L. graminifolia* × *L. marschallii*.

CROSSES BETWEEN EIGHT- AND NINE-CHROMOSOME SPECIES

Lactuca tatarica (9) × *L. bourgaei* (8) (fig. 1, *B*).—Hybrid seed was easily obtained from these 2 species when pollen of *L. bourgaei* was applied to the heads of *L. tatarica*. A population of 25 F_1 plants was grown, and all were completely self-sterile. There were some minor morphological variations among the F_1 plants.

The hybrid plants were in many respects intermediate but in general appearance resembled *Lactuca tatarica* much more than *L. bourgaei*. They did not exhibit marked hybrid vigor. Most of them attained a greater height than either parent but were not extremely large in any of their characters. All of them sprouted from the base and from the roots as does *L. tatarica*. The flower heads were a little larger than those of either parent; the corolla color was a light blue, intermediate between those of the two parents, both of which have blue flowers.

Lactuca tatarica (9) × *L. cretica* (8).—From seed developing from flowers of *L. tatarica* pollinated with pollen from *L. cretica* were obtained 17 hybrid plants, all of which were entirely self-sterile. In general appearance the hybrids resembled *L. cretica* more than *L. tatarica*. The leaves were smaller than those of *L. tatarica*, and the plants were very short-stemmed like those of *L. cretica*. None of the hybrid plants reached a height of more than a foot, and all showed marked lack of vigor. The flowers were larger than those of *L. tatarica* but did not develop the large robust heads characteristic of *L. cretica*. The spread of the corolla approximated that of *L. cretica*. The petals were a very light shade of blue, much lighter than is typical for *L. tatarica*. *L. cretica* has yellow flowers.

CROSSES BETWEEN 8- AND 17-CHROMOSOME SPECIES

Lactuca canadensis (17) × *L. bourgaei* (8) (fig. 1, *C*).—Several flower heads of *L. canadensis* were pollinated with pollen from *L. bourgaei*. Seven hybrid plants were obtained from the seed resulting from the cross pollinations, and all seven were entirely self-sterile.

The hybrid plants were intermediate between the two parents in many respects but in general appearance resembled small plants of *Lactuca canadensis* more than *L. bourgaei*. The hybrids lacked vigor, but all seven of them developed through the flowering stage. The plants reached about the same height (about 5 feet) as plants of *L. bourgaei* growing in the same greenhouse. Plants of *L. canadensis* in the same house reached a height approximately twice that of *L. bourgaei* and of the hybrids.

The flowers of the hybrids were a little larger than those of *Lactuca canadensis* and about the same shade of yellow. The yellow color of the corolla of these hybrids is contrary to expectation, since in all other interspecific hybrids studied yellow corolla color has been recessive to any shade of blue. These two species differ in many morphological characters, and distinct characteristics of both species were discernible in the hybrids. However, there is an element of doubt in regard to these hybrids.

Lactuca spicata (17) × *L. bourgaei* (8) (fig. 1, *D*).—Crosses between these two species were difficult to effect. Nine plants grown from seed obtained by applying pollen of *L. bourgaei* to washed flower heads of *L. spicata* consisted of eight selfed *L. spicata* and one large vigorous hybrid. In the rosette stage the hybrid plant showed marked

hybrid vigor. This plant seemed to have a biennial or perennial habit, as it failed to develop a seed stem during the first season; 9 months after the seed was germinated there was no evidence of stem elongation.

Lactuca graminifolia (17) × *L. bourgaei* (8) (fig. 1, *E*).—From flower heads of *L. graminifolia* pollinated with pollen from *L. bourgaei*, 31 seeds were obtained. In a population of F_1 plants from the 31 seeds there were 13 that were obviously selfed and 13 hybrids, of which 11 appeared to be normal and 2 were weak abnormal plants that died in the seedling stage. Five seeds failed to germinate. The 11 hybrid plants that appeared to be normal were grown to maturity, and all were completely self-sterile.

The hybrid plants showed some hybrid vigor, reaching a height of about 7 feet, while the *Lactuca graminifolia* plants in the same environment averaged about 2½ feet and the *L. bourgaei* plants about 4 feet. In general appearance the hybrids resembled *L. graminifolia* much more than *L. bourgaei*. In size the flowers were intermediate between those of the two parent species. The color of the corollas was a shade of bluish purple, which is intermediate between the corolla colors of the two parents.

Lactuca floridana (17) × *L. marschallii* (8) (fig. 1, *F*).—Five seeds were obtained from flower heads of *L. floridana* to which pollen of *L. marschallii* was applied. All but one of these proved to be selfed *L. floridana*. The one hybrid was a fairly vigorous plant that reached a height of 5½ feet at maturity. In general appearance it resembled *L. floridana*. Plants of *L. floridana* reached a height of about 7 feet and those of *L. marschallii* about 4 feet in the same environment in which the hybrid was grown. The flowers were intermediate in size, and the color of the corolla was blue, intermediate between those of the parents. The hybrid was completely self-sterile.

Lactuca graminifolia (17) × *L. marschallii* (8) (fig. 1, *G*).—Crosses between these 2 species were easily effected when pollen of *L. marschallii* was applied to washed stigmas of *L. graminifolia*. Thirty-two normal hybrids were obtained from an F_1 population of 44 plants. There were no weak freakish hybrids from this cross. Eleven of the 32 hybrids were grown to maturity, and all were completely self-sterile.

The hybrids were quite uniform in appearance and resembled the *Lactuca graminifolia* parent more than *L. marschallii*. These plants reached an average height of about 7 feet as compared with 4 feet for *L. marschallii* and 2½ feet for *L. graminifolia* plants in the same environment. The flowers were intermediate in size. The corollas were a bluish purple, intermediate between those of the two parents, both of which are blue-flowered.

DISCUSSION

One of the contributions of the present study is the revelation of the compatibility of the chromosome complements of certain of the 8-chromosome species with the complements of at least 1 of the 9-chromosome species and of 4 of the 17-chromosome species of *Lactuca*. Probably the most important fact demonstrated is the compatibility relationship shown to exist between the 9-chromosome species *L. tatarica*, a member of the *indica* group, and the two 8-chromosome

species *L. bourgaei* and *L. cretica*. These last 2 species are generally classified taxonomically in separate subgenera, *Mulgedium* and *Serriola*, respectively.

Although the F_1 hybrids of *Lactuca tatarica* × *L. bourgaei* and *L. tatarica* × *L. cretica* were in both cases completely self-sterile, the development of F_1 hybrids that grew through to flower production as quite normal plants shows that the genoms of at least some of the eight- and nine-chromosome species of *Lactuca* are compatible enough to function harmoniously throughout somatic development.

It has been reported[6] that the 9-chromosome *Lactuca tatarica* differs from the 8-chromosome forms *L. bourgaei* and *L. marschallii* chiefly in the addition of a small pair of medianly constricted chromosomes. Root-tip smears of both of the hybrids showed 17 somatic chromosomes. This is substantial evidence in support of the generally accepted hypothesis that the 17 chromosome species of the genus *Lactuca* had their genesis in hybrids between 8- and 9-chromosome forms.

If some condition should cause chromosome doubling in either of these 2 hybrids, an artificial 17-chromosome form would result. It is not suggested that the species employed in obtaining the 2 abovementioned hybrids are necessarily the forms that may have entered into the origin of the present 17-chromosome species. Neither of the hybrids obtained between 8- and 9-chromosome species closely resemble any of the 17-chromosome species with which the writer is acquainted. Although the species used are probably not the ones most likely to have entered into the origin of the 17-chromosome species, the compatibility shown by the development of normal F_1 hybrid plants indicates the possibility of the 17-chromosome forms, having come into existence through such hybrids.

In the previous paper[7] it was shown that compatibility exists between certain of the 9-chromosome species of the *indica* group and some of the 17-chromosome species. The results of the present studies show that compatibility relationship also exists between some of the 8-chromosome species and these same 17-chromosome forms, *Lactuca canadensis*, *L. spicata*, *L. graminifolia*, and *L. floridana*. Normal hybrid plants were obtained from crosses of *L. bourgaei* with *L. canadensis*, *L. spicata*, and *L. graminifolia* and from crosses of *L. marschallii* with *L. graminifolia* and *L. floridana*. In all of the 5 crosses the 17-chromosome form was used as the maternal parent.

In the previous paper[7] were presented data that showed a close relationship between the 4 above-named 17-chromosome species. Additional data have been recorded since that report. Partly fertile lines have been obtained from the following crosses: *Lactuca canadensis* × *L. graminifolia*, *L. canadensis* × *L. spicata*, *L. canadensis* × *L. floridana*, *L. graminifolia* × *L. floridana*, and a 3-species hybrid in which an F_3 selection from the *L. canadensis* × *L. floridana* hybrids was crossed onto *L. graminifolia*. The first 2 and the last of these 5 hybrids are almost completely self-fertile. The other 2 are only partly fertile. These results indicate a closer relationship between these 4 species than their taxonomic classification might suggest,

[6] BABCOCK, E. B., STEBBINS, G. L., JR., and JENKINS, J. A. CHROMOSOMES AND PHYLOGENY IN GENERA OF THE CREPIDINEAE. Cytologia Fujii Jubilaei Volumen, pt. 1, pp. 188-210, illus. 1937.

[7] See footnote 3.

since *L. canadensis* and *L. graminifolia* are placed in the subgenus *Serriola* and *L. spicata* and *L. floridana* in the subgenus *Mulgedium*.

Many attempts to cross the eight-chromosome species *Lactuca bourgaei*, *L. marschallii*, and *L. cretica* with the nine-chromosome species of the *serriola* group failed. The results obtained indicate that these three eight-chromosome forms are more closely related to the nine-chromosome species of the *indica* group than to the nine chromosome species of the *serriola* group.

The data presented in this and the previous paper support the following conclusions: The serriolalike 9-chromosome species *Lactuca serriola*, *L. virosa*, *L. altaica*, *L. saligna*, *L. sativa*, and closely related species constitute a compatibility group not closely related genetically to the 9-chromosome species of the *indica* group, *L. indica*, *L. squarrosa*, *L. tatarica*, and *L. raddeana*, or to any of the 8- and 17-chromosome species studied. This group of species seems to stand apart from the other groups of species. The species of the *indica* group listed above form a compatibility group among themselves and show compatibility with some of the 8- and 17-chromosome species studied.

The results of these matings show that the genoms of the 8-chromosome *Lactuca bourgaei* and the 9-chromosome *L. tatarica* are compatible to the extent of acting harmoniously together in the somatic development of hybrids between the 2 species. Moreover the genoms of each of these 2 species are capable of existing harmoniously in the somatic development of hybrids between each of them and at least three of the four 17-chromosome species studied, *L. canadensis*, *L. spicata*, and *L. graminifolia*. *L. tatarica* was also successfully mated with *L. floridana*.

No hybrids were obtained from any of the attempted crosses between the 8-chromosome species *Lactuca capensis* and species of the 8-, 9-, and 17-chromosome groups.

SUMMARY

The results are presented from 69 attempted crosses between 8-, 9-, and 17-chromosome species of *Lactuca*; 25 of these were reciprocal crosses.

Only 8 of the 44 one-way matings resulted in hybrids and only 1 of these *Lactuca bourgaei* × *L. marschallii* was fertile. No fertile hybrids were obtained from matings of 8-chromosome species with species of a different chromosome number, with the possible exception of *L. spicata* × *L. bourgaei*.

Somatic compatibility was found to exist between the chromosome complements of the 8-chromosome species when crossed with certain 9-chromosome and with certain 17-chromosome species.

Further evidence is presented to show that the *serriola* group, to which the cultivated form *Lactuca sativa* belongs, constitutes a compatibility group not closely related to the other groups of species studied.

Successful crosses between certain 8- and 9-chromosome species, although resulting in sterile hybrids, present further evidence in support of the hypothesis that the 17-chromosome species have come into existence through hybrids between 8- and 9-chromosome species.

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EFFECT OF SOIL AND PEAT ADMIXTURES ON THE GROWTH OF PLANTS IN QUARTZ SAND [1]

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INTRODUCTION

In a previous publication (*3*)[3] it was shown that millet (*Setaria italica* (L.) Beauv.) does not grow so well in pure quartz sand as in quartz sand to which a little soil is added. Experiments indicated that the beneficial effect of the soil admixture is not due to supplying a trace element, to counteracting an injurious impurity in the sand, to modifying the texture of the sand, or to reducing a total salt injury.

It was suggested that the beneficial effect of the soil is due to its buffer capacity and also to its capacity for supplying available iron. It seemed that the soil admixture should tend to prevent an injurious degree of acidity from developing in the layer immediately contiguous to the roots when the nutrient salts are such that the plant absorbs more cation than anion equivalents. In case the nutrient salts are such that the plant absorbs more anion than cation equivalents, and an alkaline reaction develops, the soil material should reduce the alkalinity and supply available iron.

Since no attempt was made to determine the hydrogen-ion concentration in the water film immediately contiguous to the root, this explanation was not based on data directly determined. The idea was suggested by the results obtained when plants were grown in pure sand with mixtures of salts containing various proportions of nitrate and ammonium salts. As the proportion of ammonium in the fertilizer was increased, the acidity increased, and the yields declined in pure quartz sand but not in the sand-soil mixtures. When all the nitrogen was supplied as nitrate, an alkaline reaction developed in quartz sand, and the plants became chlorotic, owing to a reduced absorption of iron.

Hydrogen-ion determinations made at the end of the growth period on samples representative of all the material in the pot did not always support the conclusion that the soil addition acts beneficially by creating a more favorable hydrogen-ion concentration; since some sand-soil mixtures were more acid than the pure sand cultures. However, it was pointed out that determinations of this character probably do not show the hydrogen-ion concentrations of the absorption films. Since the acidity or alkalinity is developed in the films by the non-equivalent absorption of cations and anions, there should be periods when the hydrogen-ion concentration in the films should be much higher or lower than that obtaining in the medium as a whole.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 64.

Since the foregoing explanation was suggested, additional sand-culture experiments have been conducted that throw some light on the beneficial effect of soil admixtures. These experiments are described here.

METHODS

Details regarding the pot experiments were as follows, except as otherwise noted. Glazed earthenware crocks without drainage holes were used as containers. They were of 1-gallon capacity and held approximately 5,000 gm. of quartz sand or sand-soil mixture. Ten millet, or six or seven wheat (*Triticum aestivum* L.), plants were grown per pot for periods that ranged from 25 to 50 days, according to the season. When the millet plants were cut they were in the joint stage or the heads were about to appear. The water content of the sand or sand-soil mixture, determined by weighing, was maintained at 15 percent by the addition of distilled water. The quantity of soil mixed with the sand varied with the colloid content of the soil. Unless specified otherwise, the soil addition was sufficient to supply 50 gm. of colloidal material per pot. The different mixtures of nutrient salts used in the following experiments are shown in table 1. Most data on the effect of soil admixtures were obtained with the standard mixture, No. 3. Nutrient salts, dissolved in 750 cc. of water to bring the sand to a 15-percent water content, were added just before planting. The quartz sand used in the experiments was made up chiefly of particles 1.0 to 0.5 mm. in diameter.

TABLE 1.—*Mixtures of nutrient salts used in sand cultures*

Salt used in nutrient mixture	Amount of salt applied per pot in mixture No.—				
	1	2	3	4	5
	Gram	*Gram*	*Gram*	*Gram*	*Gram*
Potassium nitrate, KNO_3			0.93	0.93	0.499
Ammonium sulfate, $(NH_4)_2SO_4$	0.938		.33		
Ammonium nitrate, NH_4NO_3		0.57		.20	
Calcium nitrate, $Ca(NO_3)_2.4H_2O$					1.103
Potassium sulfate, K_2SO_4		.82			
Potassium chloride, KCl	.685				
Magnesium sulfate, $MgSO_4.7H_2O$				.45	.45
Magnesium chloride, $MgCl_2.6H_2O$	.42	.42	.42		
Monocalcium phosphate, $Ca(H_2PO_4)_2.H_2O$	.36	.36	.36	.36	
Dipotassium phosphate, K_2HPO_4					.25
Monopotassium phosphate, KH_2PO_4					.19
Sodium chloride, NaCl	.05	.05	.05	.05	.05
Ferric tartrate, $Fe_2(C_4H_4O_6)_3.H_2O$	.0185	.0185	.0185	.0185	.0185
Manganese sulfate, $MnSO_4.2H_2O$	.0015	.0015	.0015	.0015	.0015
Boric acid, H_3BO_3	.003	.003	.003	.003	.003
Zinc chloride, $ZnCl_2$	.00013	.00013	.00013	.00013	.00013
Copper sulfate, $CuSO_4.5H_2O$	.00047	.00047	.00047	.00047	.00047

COMPARATIVE YIELDS OF MILLET IN QUARTZ SAND AND IN A SAND-PEAT MIXTURE, AS INFLUENCED BY THE HYDROGEN-ION CONCENTRATION OF THE PEAT

According to the ideas advanced, addition of soil to a quartz sand culture improves growth only through reducing the hydrogen-ion concentration where the nutrient salts are such that acidity is developed. If this is correct, sand-soil mixtures containing nearly neutral soils should exceed the yields of pure sand cultures by greater amounts than sand-soil mixtures containing acid soils of low buffer capacity.

This was true for the most part, although the correlation between the hydrogen-ion concentration of a soil and the magnitude of the increase it produced was by no means close. Probably there would have been a closer correlation if the data for different soils had been obtained in a single experiment instead of in many different experiments, since it was observed that the beneficial effect of a single soil varied considerably in different experiments, owing presumably to different conditions of light, temperature, and humidity.

The following experiment shows that the beneficial effect of the soil admixture may be influenced markedly by the hydrogen-ion concentration of the material. The experiment was conducted with peat, but previous work has shown that peat has the same growth-promoting effect as soil when added to sand cultures. In this experiment the effect of an unlimed moss peat of pH 3.5 was compared with the effects of three lots of the same peat limed some months previously to pH values of 5.0, 5.9, and 6.6. The quantities of calcium hydroxide required to produce these hydrogen-ion concentrations were, respectively, 2.9, 4.3, and 5.3 percent of the weight of peat. Before the peats were mixed with the sand they were ground to pass through a ½-mm. sieve. The nutrient salts applied to the pure sand and the sand-peat mixtures were the standard fertilizer mixture, No. 3 of table 1, used in previous experiments. Millet was grown from April 19 to May 13.

It is evident from the data in table 2 that the effect of peat on growth in sand cultures varied with the degree to which the culture had been neutralized, at least to a limit of about pH 6. If no determinations had been made of the hydrogen-ion concentrations of the cultures before and after growth, it would have seemed obvious that the effect of peat on growth in sand cultures lies in its effect on the hydrogen-ion concentration of the medium. However, the fact that the pure sand cultures before and after growth had the same pH values as the superior culture containing the peat of pH 5.9 seems to indicate that the beneficial effect of the peat does not lie in modifying the hydrogen-ion concentration of the medium.

TABLE 2.—*Effects of peats having different hydrogen-ion concentrations on the yield of millet in quartz sand*

Peat mixed with the sand: Amount (grams)	Peat mixed with the sand: pH	Air-dry yield of individual pots				Average air-dry yield per pot	Hydrogen-ion concentration of medium: At start of experiment	Hydrogen-ion concentration of medium: At end of experiment
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*pH*	*pH*
0	--------	5.12	4.06	4.42	4.07	4.42	6.3	5.6
5	3.5	2.50	2.80	4.44	3.70	3.36	5.3	4.4
10	3.5	2.28	3.27	1.58	1.80	2.23	4.6	--------
20	3.5	1.35	1.13	1.32	.96	1.19	4.4	--------
40	3.5	.46	.68	.48	.73	.59	4.2	4.4
5	5.0	5.78	5.62	5.15	6.23	5.70	5.9	5.0
10	5.0	5.43	5.82	6.15	5.76	5.79	5.7	--------
20	5.0	5.82	5.87	5.84	5.79	5.83	5.7	--------
40	5.0	5.79	5.56	5.29	5.47	5.53	5.7	5.0
5	5.9	6.50	6.58	6.73	6.65	6.62	6.3	5.4
10	5.9	6.98	6.52	6.60	7.02	6.78	6.0	--------
20	5.9	7.48	6.79	6.85	7.17	7.07	6.0	--------
40	5.9	6.73	6.32	6.92	7.23	6.80	6.0	5.7
5	6.6	6.00	6.10	6.88	5.70	6.17	6.3	5.4
10	6.6	6.34	6.83	5.88	5.99	6.26	6.6	--------
20	6.6	6.79	6.70	7.23	7.34	7.02	6.6	--------
40	6.6	6.93	7.00	6.98	6.21	6.78	6.6	6.1

Similarly contradictory evidence regarding the hydrogen-ion concentration as a factor in the increased growth produced by soil admixtures was obtained in previous work (*3*) with some of the 60 different soils used. This contradiction is disposed of in the explanation outlined in the introduction, on the grounds that the pH values determined at the end of an experiment indicate only the net acidity developed and do not show the acidity or alkalinity obtaining at different times in films contiguous to the roots.

Direct confirmation of the idea that the sand near the roots is different in reaction from the sand in the whole pot was obtained in the experiment with wheat. (See table 9.) After the sand in the whole pot had been sampled, the roots were removed and colorimetric determinations were made of the sand that adhered closely to the roots. This was done by dropping the indicator on a mass of roots and adhering sand. The results are shown in table 3.

TABLE 3.—*Hydrogen-ion concentration of all the sand in a pot and of sand adhering to wheat roots*

Nutrient salt mixture		Hydrogen-ion concentration of—	
No.	Form of nitrogen present	All the sand in a pot	Sand adhering to different lots of roots
		pH	*pH*
1	All as NH_4	4.4	4.2, 4.0
2	½ as NH_4	4.7	4.0, 5.0, 4.2, 4.7
3	⅓ as NH_4	5.2	6.4, 7.6, 8.4, 7.8
4	⅙ as NH_4	6.8	7.5, 8.8
5	All as NO_3	8.6	9.0, 9.2, 8.6

It will be seen that the sand near the roots was in most instances considerably more acid or alkaline than the average sample of the whole pot. Presumably, the microscopic film immediately contiguous to the roots differs from the average sample still more widely in reaction.

The hydrogen-ion determinations made on samples from pots receiving the No. 3 salt mixture are of especial interest. Evidently, here the plants had absorbed most of the ammonia and at the time of examination were drawing more heavily on the nitrates, so that the acidity previously developed was being reduced. Subsequent experiments with wheat and millet indicated that this was the case. A number of pots containing quartz sand and the No. 3 or No. 2 mixture of salts were planted at the same time and harvested at intervals for hydrogen-ion determinations of all the sand in the pot and of sand adhering to roots of the plants. Wheat was grown from December 16 to January 26 and millet from January 26 to March 2. The results are given in table 4.

Column 6 of table 4 gives the pH value for only the greater part of the sand adhering to the roots in a pot. Frequently the sand adhering to individual roots and to certain parts of the same root was ½ to 1 pH unit more acid or alkaline than this "majority" value. Unplanted pots had the same hydrogen-ion concentration at the end of the experiment as at the start.

TABLE 4.—*Hydrogen-ion concentrations developed by wheat and millet at different stages of growth*

Crop	Form of nitrogen present in salt mixtures	Age of plant	Air-dry weight of plants per pot	Hydrogen-ion concentration of—	
				All the sand in a pot	Most sand adhering to roots
		Days	*Grams*	*pH*	*pH*
Wheat	⅓ as NH_4; ⅔ as NO_3	6	----------	6.5	4.8
		21	0.84	6.5	6.3
		27	.87	6.8	7.5
		34	2.19	6.9	7.2
		41	3.35	7.0	7.6
Millet	⅓ as NH_4; ⅔ as NO_3	11	.04	6.9	4.2
		16	.12	6.7	3.9
		21	.47	6.4	4.6
		24	.67	6.2	5.9
		28	1.61	6.3	6.4
		32	3.13	6.5	6.2
		35	5.80	6.3	6.1
Do	½ as NH_4; ½ as NO_3	11	.03	6.9	4.2
		16	.10	6.7	3.9
		21	.36	6.4	4.6
		24	.59	6.2	4.8
		28	1.23	5.9	4.6
		32	2.25	4.8	4.8
		35	4.31	4.8	4.8

From the data in tables 3 and 4 it is concluded (1) that the sand close to the roots may have a hydrogen-ion concentration quite different from that of the whole mass of sand; (2) that the final hydrogen-ion concentration varies with the proportion of nitrate nitrogen to ammonium nitrogen; (3) that when certain proportions of ammonium and nitrate are supplied the hydrogen-ion concentration of the sand, especially that near the roots, varies with the growth of the plant, sometimes shifting from acid to alkaline.

These conclusions are supported by some previous work. Solberg (*8*) found that in quartz sand cultures the sand near the roots was about one-half of a pH unit more acid than sand between roots when nitrogen was supplied as ammonium and one-half of a pH unit more alkaline when nitrogen was supplied as nitrate. These differences are less than those recorded for millet in table 4, but the samples compared are not the same in the two cases. Nightingale (*7*) found that the hydrogen-ion concentration at the surface of apple roots sometimes differed from that of the culture solution by 1 to 2 pH units. The markedly different hydrogen-ion concentrations developed with different proportions of ammonium and nitrate nitrogen (table 3) are in general accord with the data obtained by Trelease and Trelease (*10*) on wheat grown in water cultures containing different proportions of potassium nitrate and ammonium sulfate. The hydrogen-ion concentrations did not vary so widely in the Treleases' culture solutions as in the sand cultures shown in tables 3 and 4, but this difference is probably due to the fact that the water cultures were renewed every 8 days.

Data in table 4, showing a change in reaction from acid to alkaline during the growth period, find only slight confirmation in the Treleases' water-culture data. In their work a reversal in the trend of hydrogen-ion development is shown only by the solution containing 5 parts of ammonium to 95 parts of nitrate, which was tested each

day for the last 8-day period. Probably a similar reversal would have been shown by solutions containing a higher proportion of ammonium if the plants had been grown for a longer time in the same solution. With buckwheat grown in a culture solution containing equal proportions of ammonium and nitrate, Stahl and Shive (*9*) observed a pronounced shift from ammonium to nitrate absorption after blossoming. A change conditioned by maturity of the plant, however, would not account for the shift observed in the present studies, since it does not occur in the series grown with one-half of the nitrogen as ammonium and one-half as nitrate. Moreover, the oldest plants were cut when the heads were just starting to emerge.

EFFECT OF CALCIUM CARBONATE AND INCREASED IRON ON YIELD OF MILLET IN QUARTZ SAND

If the poor growth in pure sand with the standard salt mixture is due to acidity resulting from the absorption of more cation than anion equivalents, it might seem possible to correct the condition by an application of calcium carbonate. However, too much calcium carbonate reduces the availability of iron in the quartz sand cultures to such an extent that the plants become chlorotic. The chlorosis can be overcome to some extent by increasing the iron supply, but too much reactive iron reduces the availability of the phosphate until it becomes deficient. Increasing the phosphate in order to maintain an adequate supply tends to lower the availability of the iron. Because of these conditions it may not be possible to produce optimum conditions for growth merely by adding calcium carbonate to the sand cultures.

In the following experiment the standard mixture of nutrient salts, No. 3, was used. The quantity of precipitated calcium carbonate added to the sand cultures was only 0.5 gm. per pot, since previous work (*2*) had shown that 0.8 gm. induced chlorosis. Three quantities of ferric tartrate were tested in conjunction with this application. There were also several other treatments in this experiment. Additional increments of iron were tried with the all-nitrate mixture of salts, No. 5, since diminished growth with these salts in quartz sand was found to be due to a lack of available iron (*3*). In addition to the potassium in the standard salt mixture, potassium chloride was added to some pots, since it was thought possible that the injurious effect of the acidity developed from these salts might lie in diminishing potassium assimilation, as held by Wadleigh and Shive (*11*). If this were the case, increasing the potassium concentration might ameliorate the condition. The treatment with peat of pH 5.0 was installed as a check on the efficacy of the other treatments. In the previous experiment this peat increased the yield markedly. Millet was grown from May 12 to June 4. The results of the experiment are given in table 5.

The increase produced by calcium carbonate with the highest iron application is almost as large as the increase produced by the peat of pH 5.0, and this in turn is about equal to the average increase produced by 46 different soils in 66 comparisons (*3, p. 620*). The results, therefore, support the idea that the beneficial effect of a soil application lies in acting as a buffer and in supplying available iron.

TABLE 5.—*Effect of calcium carbonate, extra iron, and extra potassium on yield of millet in quartz sand*

Substance added to quartz sand: Nutrient salt mixture[1] No.	Additional compound: Name	Additional compound: Amount	Air-dry yield of individual pots				Average air-dry yield per pot	Relative yield	pH at end of experiment
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	
3	None	----------	3.82	3.40	4.78	4.04	4.38	100	5.9
3	-----do	----------	4.92	4.76	4.53	4.79			
3	Calcium carbonate	0.5	4.06	5.98	5.00	5.09	5.03	115	7.5
3	-----do Iron	.5 .037	3.51	5.21	6.78	6.07	5.39	123	7.5
3	Calcium carbonate Iron	.5 .148	6.80	5.35	6.68	6.10	6.23	142	7.5
3	Potassium chloride	.343	4.11	4.72	4.99	4.15	4.49	103	5.2
3	-----do	.686	3.52	4.05	4.14	3.87	3.90	89	6.5
3	-----do	1.029	3.94	3.68	4.10	3.58	3.83	87	6.5
3	Peat, pH 5.0	10	6.40	6.68	6.30	--------	6.46	147	4.7
3	Peat Potassium chloride	10 .343	6.00	5.82	5.45	5.62	5.72	131	4.7
5	None	----------	3.31	4.54	3.91	2.60	3.59	82	7.8
5	Iron	.037	5.18	5.15	4.25	4.25	4.71	108	7.5
5	-----do	.148	4.19	4.41	4.62	3.96	4.30	98	8.0

[1] Nitrogen present in nutrient salt mixtures: In No. 3, 1/3 N as NH_4, and 2/3 N as NO_3; in No. 5, all N as NO_3.

The failure of the extra potassium applications to increase growth indicated that a potassium deficiency is not the cause of reduced growth in the pure sand cultures.

In pots receiving the all-nitrate salt mixture, all plants were markedly chlorotic during early growth, but toward the end of the experiment the color improved considerably and at that time the plants receiving extra iron were an almost normal green. The improved color at the later period when the heads were forming may be connected with an increase in the plants' requirement for potassium as compared with nitrogen, since an increase of this kind, if sufficient, would develop an acid reaction instead of an alkaline one in the absorption zone. The appearance of the plants and the yields indicated that the extra iron applications increased iron assimilation slightly but not to an optimum degree when this mixture of nutrient salts was applied.

COMPARATIVE YIELDS OF MILLET IN QUARTZ SAND AND IN SAND-SOIL OR SAND-PEAT MIXTURES, AS INFLUENCED BY THE WATER CONTENT AND BY FLUSHING

According to the ideas outlined in the introduction, unfavorable conditions for growth in pure sand cultures are developed in the zone of root absorption and do not necessarily obtain throughout the whole medium. It was thought that if the absorption zone could be flushed at intervals with the salt solution draining from the whole pot, growth should be as good in quartz sand as in sand-soil mixtures. The first experiment in which the pots were drained and flushed at intervals is described in the following paragraphs. This experiment was also designed to test an idea that had been expressed that better growth would be obtained in pots provided with drainage holes than in the standard closed crocks.

The pots used in this experiment were especially designed for drainage and held approximately 8,000 gm. of sand. The drainage holes of some pots were stopped with cotton and rubber cement, while the holes of other pots were left open. Half the pots were filled with quartz sand and half with a sand-Marshall soil mixture containing 1 percent of colloidal material. Most pots were maintained at a water content of 15 percent, but water was added to other pots until approximately 500 cc. percolated through. The 500 cc. of percolated solution was added to the pots at the next watering along with sufficient water to again provide a percolate of about 500 cc. For the 15 percent water content, 1,175 cc. of water was required, and for the drained pots, 2,763 cc. of water was required to produce the percolate of 500 cc. The sand and sand-soil mixtures in the drained pots thus held approximately 27 percent of water after drainage. The drained pots were filtered 11 times during the 27 days the experiment lasted. The standard mixture of nutrient salts, No. 3 of table 1, was applied to all pots. The quantity of salts was increased to eight-fifths of the normal quantity, however, since the pots in this experiment held approximately 8,000 gm. of sand instead of the usual 5,000 gm. Millet was grown from September 29 to October 26. The results of the experiment are shown in table 6.

TABLE 6.—*Effect of water content and drainage on yield of millet in quartz sand and a sand-soil mixture*

Material in pots	Kind of pots	Water content	Air-dry yield of individual pots			Average air-dry yield per pot
		Percent	*Grams*	*Grams*	*Grams*	*Grams*
Quartz sand only	No holes	15	6.47	4.48	5.47	5.47
Do	Holes	15	5.74	5.80	5.27	5.60
Do	do	27+	5.76	6.86	7.52	6.71
Sand plus Marshall soil	No holes	15	9.86	10.78	10.01	10.22
Do	Holes	15	10.78	9.90	10.41	10.36
Do	do	27+	9.00	7.56		8.28

It will be seen that the pure quartz sand and the sand-soil mixture, maintained at a water content of 15 percent, gave almost exactly the same yield in closed pots as in pots provided with holes in the bottom. Thus it is evident that the added aeration afforded by drainage holes does not improve growth conditions when the cultures are maintained at a favorable water content. The quartz sand culture flushed with excess water yielded about 20 percent more than the quartz sand of 15 percent water content; whereas the flushed sand-soil mixture yielded about 20 percent less than the same culture with 15 percent of water. The conclusion here is not certain, since the excess-water cultures differed from the standard cultures in three respects: (1) The excess-water cultures contained a less concentrated salt solution than the standard cultures, since the salts were dissolved in 2,763 cc. of water instead of in 1,175 cc.; (2) the pore space in the excess-water cultures remained filled with water after drainage, whereas the pore space in the standard cultures was little more than half filled by water; (3) the excess-water cultures were flushed 11 times during the experiment.

It is believed that in the case of the sand-soil mixtures the slightly

poorer growth in the flushed series than in the standard series was due to the second of the differences just mentioned. When the pore space is filled with water the decomposition of organic matter in the soil may be expected to produce unfavorable conditions. In the case of the pure quartz sand cultures, the slightly better growth in the flushed pots is believed to be due to the third difference. The flushing tended to remove the injurious conditions developed by the roots in the zone of absorption.

These conclusions seem little more than guesses when based on the data of this one experiment. They are, however, substantiated by other data. In connection with the results of the following experiment it is important to bear in mind that in this particular experiment any one or all three of the differences mentioned are responsible for only a slightly increased growth in the pure sand cultures.

It was thought that in the foregoing experiment the pots were not flushed frequently enough to maintain the solution in contact with the roots at approximately the same composition as the solution in the whole pot. Two more experiments were conducted in which the flushing was much more frequent.

In these experiments the regular 1-gallon crocks with drainage holes drilled in the bottom were employed. The all-ammonia mixture of nutrient salts, No. 1 of table 1, was used instead of the standard mixture, in order to provide a more severe test of the efficacy of flushing. With this mixture of salts, growth of millet in quartz sand cultures is especially poor, being only about one-fifth of that obtained in good sand-soil or sand-peat mixtures. Automatic flushing was provided by a siphon dripping from a 5-gallon bottle into a Soxhlet tube. The Soxhlet tube emptied 200 cc. of solution into the pot every hour or two. Such a large volume of water was required for flushing with this apparatus that distilled water could not be used without inordinately reducing the salt concentration in the pot; hence a salt solution was used that contained for every 1,400 cc. of water the quantity of salts applied to one pot. The solution was adjusted to pH 5.5 with sodium hydroxide. As a check on the effect of frequent flushing, some pots were made up with 1,400 cc. of water, which was the maximum amount a pot would hold after excess water had been added and allowed to drain away. After the plants had attained some size, these pots were flushed every 2 days with 200 to 500 cc. of the same solution as that applied to the frequently flushed pots. As a check on the efficacy of flushing, a series of pots was installed with a sand-peat mixture. The peat was a commercial material used for soil improvement and had been limed to a pH of 5.5. It was mixed with the sand in the proportion of 25 gm. of peat to approximately 5,000 gm. of sand.

Automatic flushing could not be started until the plants in the sand were about 1½ inches tall and those in the sand-peat mixture were somewhat taller. The effect of flushing the pots as soon as the plants were up was tried, but the solution burned the leaves badly. In the first experiment the plants were grown from October 24 to December 12 and in the second from January 24 to March 17. The greater growth in the second experiment was due to the more favorable light conditions obtaining in the second period. The results of the two experiments are shown in table 7.

TABLE 7.—*Effect of frequent flushing on yield of millet in quartz sand and in a sand-peat mixture*

Material in pots	Water content of pots	First experiment			Second experiment		
		Air-dry yield of individual pots		Average air-dry yield per pot	Air-dry yield of individual pots		Average air-dry yield per pot
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Quarts sand only	Normal, 15 percent	0.51	0.58	0.55	1.00	1.15	1.08
Do	Occasional flushing	.60	--------	.60	1.37	1.60	1.49
Do	Frequent flushing	2.06	--------	2.06	5.20	4.58	4.89
Sand plus peat	Normal, 15 percent	2.94	3.86	3.40	8.32	8.10	8.21
Do	Frequent flushing	1.66	1.29	1.48	6.45	6.92	6.69

It is apparent from the results shown in tables 6 and 7 that flushing the pots had a very different effect on pure sand cultures from that on sand cultures containing a small amount of soil or peat. Occasional flushing of the sand-soil mixture depressed the yield slightly, and frequent flushing had the same effect. In the pure sand cultures occasional flushing increased the yield only slightly, but frequent flushing increased the yield so markedly that it seems that this treatment may improve growth conditions in somewhat the same way as soil or peat admixtures. The failure of plants in frequently flushed sand to attain the growth made by plants in the sand-peat mixture is doubtless due in part to the fact that flushing could not be started until the plants were 10 or 11 days old. Possibly, also, frequent flushing did not so fully correct conditions as did the peat admixture.

It is felt that the experiments on flushing strongly support the idea that the beneficial effect of soil or peat admixtures on quartz sand cultures lies in correcting adverse conditions that develop in a zone contiguous to the roots.

COMPARATIVE YIELDS OF MILLET IN QUARTZ SAND AND IN A SAND-SOIL MIXTURE, AS INFLUENCED BY THE MECHANICAL COMPOSITION OF THE SAND

The data that have been discussed were obtained with many different lots of quartz sand, but they were all of approximately the same mechanical grade. Two-thirds of the particles were between 1.0 and 0.5 mm. in diameter. It seemed desirable to determine whether the effects of soil admixtures would be more or less pronounced with other grades of sand. An experiment was therefore begun in which coarser and finer grades of sand were compared with the standard grade. The mechanical analyses of the three kinds of sand (table 8) were made by E. F. Miles, of the Bureau of Plant Industry.

In this experiment the standard mixture of nutrient salts was used and the Spearfish soil was applied at a rate to supply 50 gm. of colloid per pot. Millet was grown from November 1 to December 13.

The results of the experiment were not very informative. The fine grade of sand, both with and without the soil admixture, was so compact that the roots penetrated it only slightly. Many of the roots formed later arched out of the sand without the tips penetrating. A mixture of 25 percent of the finer grade and 75 percent of the stand-

TABLE 8.—*Mechanical composition of three grades of quartz sand*

Diameter of particles (mm.)	Particles in sand of indicated grade		
	Coarse	Standard	Fine
	Percent	*Percent*	*Percent*
2+	27.1	0	0
2–1	68.6	16.7	0
1–0.5	3.7	64.8	.2
0.5–0.25	.5	17.4	1.5
0.25–0.1	.1	.9	84.6
0.1–0.05	0	0	13.1
0.05–0.005	0	0	.6

ard grade was likewise too compact for normal root growth. With the standard grade of sand the soil admixture produced the usual increase in growth. The average weight of plants in this pure sand was 1.61 gm. per pot, and in the sand-soil mixture the weight was 2.43 gm. With the coarser grade of sand the effects of the soil admixture were more pronounced. The pure sand yielded 0.51 gm. per pot and the sand-soil mixture 2.14 gm.

The preceding results indicate that the coarser the sand the more beneficial is the addition of soil. However, in the case of the coarse sand it is possible that part of the beneficial effect of the soil was due to improvement in the water-holding capacity of the medium. In the coarse sand without soil the plants wilted somewhat even when the water content was only slightly reduced.

COMPARATIVE YIELDS OF WHEAT IN QUARTZ SAND AND IN A SAND-SOIL MIXTURE, AS INFLUENCED BY THE MIXTURE OF NUTRIENT SALTS APPLIED

Most measurements of the increased yields produced by adding soil to sand cultures were obtained with millet, but apparently the results apply to most plants. Results similar to those shown by millet were observed with rice (*Oryza sativa* L.), white mustard (*Brassica alba* (L.) Boiss.), cotton (*Gossypium* sp.), and dwarf sunflowers (*Helianthus* sp.). Marquis wheat, on the other hand, yielded almost as much in pure quartz sand as in sand-soil mixtures in several experiments, some of which have been published *(5, 6)*.

It was thought probable that this exceptional behavior of wheat was characteristic only of the particular mixture of nutrient salts applied. An experiment was therefore conducted with a series of nutrient salt mixtures in which the source of nitrogen ranged from all nitrogen as ammonia to all nitrogen as nitrate. The composition of these salt mixtures is shown in table 1. Each of the mixtures contained practically the same quantities of nitrogen, phosphorus, potassium, magnesium, sodium, and minor nutrients, but some mixtures contained different quantities of calcium, sulfate, or chlorine. The soil added to one series of pots, at the customary rate of 50 gm. of colloid per 5,000 gm. of sand, was a sample of the Nacogdoches fine sandy loam having a pH value of 6.3. Marquis wheat was grown from December 11 to January 22. The results of the experiment are given in table 9.

Evidently the growth of wheat in sand cultures may be markedly improved by a soil admixture when the nutrient salts contain an undue

Table 9.—*Effect of different nutrient salt mixtures on yield of wheat in quartz sand and in a sand-soil mixture*

Nutrient salt mixture No.	Form of nitrogen present	Material in pots	Air-dry yield of individual pots				Average air-dry yield per pot	Yield in sand-soil culture as percentage of yield in sand	Hydrogen-ion concentration of medium at— Start of experiment	End of experiment
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*pH*	*pH*
1	All as NH_4	Sand only	1.05	0.80	0.80	0.83	0.87	100	6.0	4.4
2	½ as NH_4		2.48	2.32	2.33	2.01	2.29	100	6.0	4.7
3	⅓ as NH_4		2.37	2.27	2.37	2.11	2.28	100	6.0	5.2
4	⅙ as NH_4		2.75	2.60	2.43	2.76	2.64	100	6.0	6.8
5	All as NO_3		1.72	1.95	1.82	2.15	1.91	100	6.7	8.6
1	All as NH_4	Sand plus soil	2.20	2.13	1.96	1.92	2.05	236	6.0	4.6
2	½ as NH_4		2.82	2.40	2.98	2.64	2.71	118	6.0	5.0
3	⅓ as NH_4		2.21	2.42	2.53	2.58	2.44	107	6.0	5.8
4	⅙ as NH_4		2.28	2.44	2.60	2.72	2.51	95	6.0	6.5
5	All as NO_3		2.27	2.68	2.77	2.01	2.43	127	6.0	7.6

proportion of either essential cations or anions, as in the case of the all-ammonium and all-nitrate mixtures. But when the standard salt mixture No. 3 was used, the increase produced by soil was only 7 percent in this experiment, and in seven other comparisons involving four different soils the average increase was 9 percent. On the whole, wheat seems less sensitive than millet to conditions in quartz sand, for when the No. 3 salt mixture is used a soil admixture usually increases the growth of millet about 50 percent. And the increases produced in wheat by the application of soil with the other salt mixtures are smaller than those usually produced in millet under similar conditions.

Although wheat seems more tolerant than millet or rice of conditions obtaining in quartz sand cultures, it seems to alter the hydrogen-ion concentration of the medium to about the same extent as millet or rice, as shown by table 10.

Table 10.—*Hydrogen-ion concentration of quartz sand after growth of millet, wheat, and rice with different nutrient salts*

Nutrient salt mixture No.	Form of nitrogen present	Hydrogen-ion concentraton of quartz sand after growth of— Millet	Wheat	Rice
		pH	*pH*	*pH*
1	All as NH_4	3.8	4.4	......
2	½ as NH_4	4.3	4.7	4.4
3	⅓ as NH_4	5.5	5.2	5.5
4	⅙ as NH_4	5.8	6.8	7.3
5	All as NO_3	7.1	8.6	......

This comparison is not conclusive since the data are the results of single experiments and the hydrogen-ion concentrations developed by millet have been found to vary considerably in experiments conducted at different times. Presumably all conditions that affect the ash composition of the crops would affect the hydrogen-ion concentrations developed. It seems probable, however, from the data at hand, that modifications in the hydrogen-ion concentrations produced by the growth of wheat are not widely different from those produced by millet and rice.

RECAPITULATION OF EVIDENCE CONCERNING THE BENEFICIAL EFFECT OF SOIL ADMIXTURES IN QUARTZ SAND CULTURES

The experimental results reported in this and a previous publication (*3*) strongly support the explanation that has been given for the beneficial effect of adding soil to a quartz-sand culture. The general features of the explanation are that in pure quartz sand an unfavorable hydrogen-ion concentration is developed, owing to a nonequivalent absorption of cations and anions; that the soil admixture improves growth by acting as a buffer to the hydrogen-ion concentration developed; and that the soil also supplies available iron. A recapitulation of evidence bearing on these assumptions follows.

That the roots develop a hydrogen-ion concentration owing to the nonequivalent absorption of nutrient cations and anions was shown by experiments conducted with millet (*3*) and wheat in quartz sand. In these experiments mixtures of nutrient salts containing different proportions of ammonium and nitrate ions were compared. When nitrogen was supplied exclusively as ammonium ions, the quartz sand became plainly too acid for the best growth; and when nitrogen was supplied exclusively as nitrate, the sand became plainly too alkaline, since the plants became chlorotic, owing to an insufficient absorption of iron. When one-third of the nitrogen was supplied in the form of ammonium and two-thirds as nitrate, as in the standard salt mixture, the sand was somewhat more acid at the end of growth than at the beginning. But the acidity developed was not sufficient to curtail growth markedly, if one judged by the hydrogen-ion concentration of all the sand in a pot before and after an experiment. In fact, some sand-soil mixtures were more acid than the pure quartz sand cultures at both times of examination.

However, examination at intervals during growth of sand adhering to the roots showed that, when the standard salt mixture is used, sand close to the roots may be highly acid during early growth and nearly neutral to alkaline during later growth. It appeared, therefore, that hydrogen-ion determinations of all the sand in a pot at the start and end of an experiment showed only the net acidity or alkalinity developed, and not the conditions affecting the roots at any time. Even when the hydrogen-ion concentration of the whole pot is near the optimum, the hydrogen-ion concentration very near the roots may be unfavorable a considerable part of the time.

The difference between conditions obtaining near the roots and throughout the pot is indicated also by the appearance of the plants. Usually the poorer growth of millet in quartz sand than in sand-soil mixtures is apparent when the plants are very small, only 1 to 2 inches tall. At this stage of growth the sand in the pot as a whole could have been altered little by the plants' absorption of ions. In a zone close to the roots, however, the reaction developed could be as unfavorable as that shown by the whole pot at the end of growth when nitrogen is supplied exclusively as ammonium or as nitrate.

Further evdence that in sand cultures an unfavorable condition may develop near the roots is provided by the flushing experiments. When the pots were flushed every 2 to 3 hours, growth was markedly improved in the sand cultures but slightly depressed in the sand-soil and sand-peat mixtures. When the sand cultures were flushed every

other day, there was only slight improvement in growth. These facts are in accord with the idea that the unfavorable conditions in sand cultures are strongly localized and develop quickly.

That the soil admixture improves growth by acting as a buffer to the hydrogen-ion concentrations developed is shown by several experiments, most of which are reported in a previous publication (*3*). When the standard salt mixture developing moderate acidity was used, growth in quartz sand was improved by such diverse materials as a neutralized sodium silicate, a ferric oxide gel, activated charcoals, samples of peat having pH values above 5.0, and calcium carbonate plus ferric tartrate. All of these materials had greater or less buffer capacities, especially against acid.

That the capacity of the soil for supplying iron is also concerned in the beneficial effect of soil additions, especially when an alkaline reaction is developed, is indicated by two facts. (1) When an all-nitrate salt mixture was applied to quartz sand, the plants became chlorotic, owing to an iron deficiency. This condition was partly corrected by adding extra iron to the sand. In the soil-sand culture the plants were green and were not improved by additional iron. (2) When a small amount of calcium carbonate was added to quartz sand fertilized with the standard mixture, growth was improved by only 15 percent; but calcium carbonate and extra iron together increased growth by 42 percent.

DISCUSSION

The results obtained in this study emphasize some of the important differences in soil, water, and sand cultures. In a soil culture the solution at the start may be quite different from the salt solution originally supplied, owing to reactions with the colloidal material. This applies particularly to concentrations of hydrogen and phosphate ions. And, since the salt solution is not renewed, it should change with the growth of the plants, except as it is stabilized by the soil colloids. The solution in contact with the roots should be somewhat different from that in the medium as a whole, owing to absorption by the roots, but creation of differences in the root zones is opposed by the colloidal material.

In a water culture, the solution in the container should at the start be the same as that originally supplied, and it should alter little with growth of the plants if the solution is frequently renewed. Also, the solution in contact with the roots should be practically the same as all the solution in the container, particularly if the solution is agitated by aeration. However, there is reason for believing that the solution in the absorption zone of roots may differ from that in the body of the container in the case of water cultures in which precipitates form and adhere to the roots.

Some conditions in a quartz sand culture are midway between those obtaining in water and soil cultures and some are more extreme. In a pure quartz sand culture the solution at the start should, as in a water culture, be the same as that originally supplied. Since the solution is not renewed and is not buffered by a reactive material, it should alter with the growth of the plants more than the solution in a soil culture. Also, the solution in immediate contact with the roots should differ from that in the whole container more markedly than in the case of soil or water cultures.

This difference between the solution in the whole container and in the absorption zone varies with the rate of diffusion from and into the absorption zone. It should also vary with the kind of plant and the composition of the nutrient solution. If a plant absorbed equivalent amounts of the nutrient cations and anions, the hydrogen-ion concentration of the absorption zone would remain neutral or the same as that in the container as a whole. The most pronounced changes in the reaction of the absorption zone are brought about by varying the proportions of ammonium and nitrate ions, since this involves a change in one of the major nutrients absorbed from cation to anion. Other differences in the composition of the nutrient solution should have comparatively little effect on the reaction developed by absorption, since no other major nutrient can be supplied as either cation or anion.

It might be supposed that in sand cultures any hydrogen-ion concentration between pH 4 and 7 could be attained by properly apportioning the nitrogen between ammonium and nitrate ions. This is approximately true for the reaction of the whole medium at the end of a growth period, provided the nitrogen supply is largely utilized. It does not seem to apply, however, to sand close to the roots. Near millet and wheat roots the reaction of the sand may vary between early and later growth from markedly acid to nearly neutral or alkaline, when certain proportions of ammonium and nitrate ions are supplied; when other proportions of ammonium and nitrate are supplied the reaction may remain strongly acid during the whole period. The early acidity developed is doubtless due to the fact that most plants, especially young ones, absorb ammonium ions more rapidly than nitrate. The shift from acidity to alkalinity that sometimes occurs is probably due to a depletion of the ammonium ions.

Even the reaction of the whole culture at the end of a growth period cannot be accurately controlled by varying the proportions of ammonium to nitrate unless the total application of nitrogen is adjusted to growth conditions. Hydrogen-ion determinations have been made on all the sand in a pot after growth of the plants in 14 experiments conducted at different times of the year. In all experiments the same quantity of the 1/3-ammonium, 2/3-nitrate salt mixture was applied. The hydrogen-ion concentrations ranged from pH 4.5 to 6.2 in different experiments, and were closely correlated with the dry weights of the crop. The more acid reactions were obtained in experiments where the growth was less.

It is not certain that a root zone maintained in a wholly neutral condition would be especially favorable for growth. Under such conditions the plants might suffer from an iron deficiency. When water cultures are kept nearly neutral, special procedures have to be followed to keep the plants supplied with available iron. One procedure is to grow the plants part of the time in a phosphate-free solution, where the iron is more available. Another method is to supply the iron in organic form as ferric tartrate or ferric citrate. The solution, however, should be renewed frequently, since the iron does not remain available long even when supplied in these forms. The ferric tartrate dissolves under the influence of sunlight into a ferrous form that reacts with potassium ferricyanide, but as the solution is aerated the iron is precipitated as ferric hydroxide. A third method that has been followed by Von der Crone (*1*) and Zinzade (*12*) is to supply a large amount of iron as ferric phosphate.

It is probably not sufficiently appreciated that the reaction developed where the root is absorbing may be quite different from that of the medium as a whole. It was found by one of the authors years ago that pineapples and rice became chlorotic owing to lack of available iron in calcareous soils but did not become chlorotic in soils rendered alkaline with sodium bicarbonate (*4*). This discrepancy was not explained at the time. Since the nitrogen in these experiments was supplied as ammonium sulfate, it is probable that the plants developed sufficient acidity in the absorption zone to neutralize the sodium bicarbonate in that zone and render iron available, but not sufficient acidity to neutralize the calcium carbonate in contact with the roots. Possibly this theory may explain some of the exceptions found in the distribution of acid-loving plants.

SUMMARY

In a previous publication (*3*) it was shown that millet does not grow so well in pure quartz sand as in quartz sand to which a little soil has been added. How the soil admixture improves growth was not shown with certainty. An answer to this question is provided by data reported in the present paper.

The beneficial effect of a soil admixture with quartz sand appears to be due to the buffer capacity of the soil and to its capacity for supplying iron.

In pure quartz sand unfavorable hydrogen-ion concentrations may develop, owing to the nonequivalent absorption of cations and anions. If the nutrient salts contain all the nitrogen in the form of ammonium, the sand becomes markedly acid; and if the nitrogen is applied exclusively as nitrate, the sand becomes alkaline or so nearly neutral that absorption of iron is inhibited. If nitrogen is supplied partly as ammonium and partly as a nitrate, the reaction is markedly acid at first, owing to ammonium ions being absorbed more rapidly than nitrate ions; later, the reaction developed may be nearly neutral or alkaline if the ammonium ions are sufficiently depleted. This occurred in experiments in which a salt mixture was used that contained one-third of the nitrogen as ammonium and two-thirds as nitrate. In this case hydrogen-ion determinations made on all the sand in a pot at the end of growth showed only the net acidity developed and did not show conditions existing near the root at different times during growth. Sand adhering to the roots may differ widely in reaction from all the sand in a pot. In sand cultures containing soil admixtures, the development of unfavorable reactions in the zone of root absorption is opposed by the soil colloids. The soil colloids may also improve growth by supplying iron in case a nearly neutral or alkaline reaction is developed.

Differences in soil, sand, and water cultures as mediums for growth are pointed out, and the importance of considering the hydrogen-ion concentration of the zone contiguous to the root, rather than that of the whole medium, is emphasized.

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STUDIES OF SOME FACTORS AFFECTING FRUIT SETTING IN SOLANUM TUBEROSUM IN THE FIELD IN LOUISIANA [1]

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INTRODUCTION

Nonfruitfulness of the potato (*Solanum tuberosum* L.) greatly complicates improvement through breeding. Location of the breeding operations in areas found by experience to be favorable for fruit setting will partly eliminate the handicap. However, the limited number of these areas places a serious restriction on the amount of breeding work that can be done.

Nonfruitfulness is due mainly to pollen sterility in many varieties or to premature abscission of flowers before fertilization can occur. These handicaps greatly complicate the problem of improvement through breeding by increasing the amount of labor necessary to obtain seedling populations, by limiting the size of many progenies, and by making certain crosses impossible so that many combinations cannot be tested.

In most localities where potato breeding is under way, more attention has been given to nonfruitfulness due to pollen sterility than to that resulting from premature abscission of flowers. However, under controlled conditions in Louisiana, the latter constitutes as great a handicap as lack of viable pollen, or even greater. This is true probably because environmental conditions are frequently unfavorable for fruit setting in bags under field conditions in Louisiana.

Because pollen sterility and premature flower shedding are to a great extent genetically controlled, data showing the degree of both conditions in all varieties used as breeding material should have practical value. Therefore, these investigations were made to study (1) the extent of pollen sterility in named and seedling varieties, (2) the effect of bagging of flowers on fruit set, (3) the effect of stage of development and number of pollinations on fruit set, and (4) the effect of some natural environmental conditions on fruit set.

EXPERIMENTAL DATA

EXTENT OF POLLEN STERILITY IN NAMED AND SEEDLING VARIETIES

Stuart (*8*)[2] made the following statement with reference to pollen sterility in potatoes:

> * * * In plant breeding studies extending over a period of sixteen years it has been the writer's privilege to examine the stamens of a great many varieties, and in but few instances has an abundance of viable pollen been found. The data show a very much larger proportion of foreign varieties producing viable pollen than of American varieties. Of the many varieties studied * * * only

[1] Received for publication May 25, 1942. Cooperative investigations with the Department of Horticulture, Louisiana Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 75.

four were noted which could be regarded as dependable sources from which to obtain viable pollen.

Krantz and Hutchins (*4*) credited the prevalence of nonfruitfulness in the potato, due to flower abscission and pollen sterility, with being primarily responsible for the paucity of potato-breeding work. Stevenson and Clark (*7*) stated:

> Sterility, or lack of fruitfulness, which is very generally present in potato varieties, is the source of the greatest difficulty in sexual breeding, and in spite of much study of the condition it remains the greatest handicap of the potato breeder.

In order to obtain an estimate of the amount of viable pollen present in some varieties being used in potato breeding, percentages of pollen stainable in acetocarmine were calculated during the flowering seasons of 1940 and 1941. Percentages, based on counts of 100 grains from each of 3 flowers, and the relative amount of pollen per flower were determined for each variety.

Pollen grains of potato that absorb the acetocarmine stain have a nonstaining exine, are spherical in outline, and usually have four equidistant germ pores, or small openings, through which the pollen tubes emerge. The cytoplasm stains a uniform, deep red and does not always have the same shape as the exine; it may vary from spherical to almost square with cornerlike projections immediately beneath the germ pores. The cytoplasm of normal grains is very finely granular. These stainable grains may vary considerably in size within a clone. Pollen classed as abortive may vary from shrunken, wrinkled, hyaline exines without any trace of cytoplasm to grains normal in shape and size but having numerous lightly staining or nonstaining alveolate or vacuolelike structures embedded in the differentially staining cytoplasm.

The stainability and relative abundance of pollen of 36 varieties and seedling varieties are presented in table 1. From these data it appears that the higher percentages of pollen stainable are usually associated with relatively large amounts of pollen grains. From experience, it appears that only varieties having a medium or abundant quantity of pollen and with at least 30 percent of it stainable can be used successfully as pollen parents. With a knowledge of the percentage of pollen stainable, a breeding program can be planned with a minimum of wasted effort. Approximately two-thirds of the varieties listed in table 1, representing some of the important breeding material in the United States, cannot satisfactorily be used as male parents. Obviously, hybridization is seriously limited by the scarcity of genetically valuable parents that produce good pollen.

EFFECT OF BAGGING OF FLOWERS ON FRUIT SET

A few reports on the influence of flower isolation and type of isolator on fruit set of the potato are available in the literature. Salaman (*6*) and East (*2*) considered the covering of potato flowers with bags as unnecessary and likely to cause injury to the delicate style. The former worker stated that "when bagged, the flower invariably drops." East (*2*) implied that shutting out light and preventing air circulation by bagging reduced the percentage of fruit set.

TABLE 1.—*Stainability and relative abundance of pollen in varieties and seedling varieties of potatoes tested at Baton Rouge, La., 1940 and 1941*

Variety or seedling	Pollen stainable		Relative amount of pollen
	1940	1941	
	Percent	*Percent*	
Ackersegen	16.0		Very scarce.
Albion	25.5	8.3	Medium.
Arnica [1]	45.3		Do.
Chippewa	11.0	7.5	Scarce.
Earlaine [1]	62.5	42.7	Abundant.
Early Rose	12.5	1.0	Very scarce.
Green Mountain	16.1		Medium.
Hindenburg [1]	51.6	44.0	Do.
Houma	6.7	5.0	Very scarce.
Irish Cobbler		3.7	Scarce.
Katahdin [1]	72.0	56.7	Abundant.
Pennigan	16.0		Very scarce.
Pontiac		45.0	Scarce.
Richter Jubel	34.0	20.0	Medium.
Sebago	16.6	3.0	Scarce.
Sebec [1]		70.7	Abundant.
Sequoia	38.1	[2]	Scarce.
Shamrock [1]	45.5	45.5	Medium to abundant.
Triumf [1]		38.7	Medium.
Triumph	8.7	6.7	Very scarce.
S45941 [1]	55.3		Medium.
S46000	9.5		Very scarce.
S46442 [1]	59.1	31.0	Medium.
S46244		11.3	Abundant.
S46926 [1]	46.0		Medium to abundant.
S47140	73.0		Very scarce.
S47142		5.0	Do.
S47148	25.3		Scarce to medium.
S47182 [1]		31.0	Medium.
S47194		1.3	Medium to abundant.
S47402	19.5		Scarce to medium.
B-127	26.4	23.0	Medium.
X570-84 [1]	32.3	40.0	Abundant.
X528-34		3.0	Scarce.
X528-170 [1]		46.0	Abundant.
X528-175		4.5	Medium.

[1] Variety that can be feasibly used as pollen parent.
[2] No pollen.

However, Stuart (*8*) stated:

While it is true that the pistil of the flower is easily broken off, and that few insects visit the flowers, it is not necessarily true that the inclosure of the emasculated flowers in paper bags causes any more of them to drop off than if left uncovered, provided the operator follows the suggestion given relative to including as much foliage with the flowers as is possible.

And Lunden (*5*) stated: [3]

For the control of pollination sometimes parchment bags and sometimes manila bags were used. Where it has been difficult to get a good development of the tops in small closed bags, large bags were used, enclosing part of the upper leaves in the bag. In this way the danger of foreign pollination is practically excluded, while giving a chance for some ventilation. This method has given good results.

Uspenskii (*9*) listed three methods of isolation in general use in Russia, namely, enclosing in cloth or parchment bags and covering the pollinated stigma by a short piece of straw tube. He reported that isolation on the whole tends to lower the percentage of fruit set as compared with that of nonisolated controls. Of the three kinds of isolation, parchment bags gave the best fruit set, and the straw isolators gave the lowest percentage of seed. However, when pollinations were made during rainy weather, the straw isolators ranked

[3] From translation by Margareta Ahlquist; made under the supervision of F. A. Krantz, Division of Horticulture, Minnesota Agricultural Experiment Station. [Processed.] See p. 25.

highest in percentage of fruit set and parchment bags ranked higher than the unbagged controls. He explained this difference by the fact that isolators protect the stigma from rain.

Stevenson and Clark (7) stated that in the potato-breeding work of the United States Department of Agriculture at Beltsville, Md., the emasculated and pollinated inflorescence is enclosed in a 1-pound paper bag.

In the present work the fruit set under two kinds of bags, kraft-paper bags and cheesecloth bags stretched over wire frames, was tested in comparison with that of nonbagged controls in 1940. In 1941 only kraft-paper bags were used.

Plants that had several buds per cyme in the proper stage for making pollinations were selected. Only buds that would have opened normally within the next 2 days were chosen for the test, and all open flowers and immature buds on the selected cymes were removed. Usually three to six buds per flower cluster were left for pollination. On these buds about three-fourths of each calyx and corolla was removed with a pair of sharp-pointed tweezers, exposing the stamens and pistil. Emasculation was accomplished by placing the point of the tweezers against the inner surface of the firm fleshy anthers and pushing outward. In this manner the anthers were broken off just below their base with the least amount of injury to the slender, easily broken pistil. Furthermore, this method eliminated the danger of leaving portions of anthers, which occurs often when the anthers are pulled away. Immediately after emasculation the flowers were pollinated by holding them at the base and dipping the stigma into a mass of fresh pollen collected in a small shallow container.

In each case a small amount of foliage was enclosed in the bag with the cyme. This practice has been found to increase the percentage of fruit set, probably because the leaves maintain higher humidity and lower temperatures in the bags than would be the case without them. A small cardboard tag on which all essential information was recorded was attached to the stem by a string. After pollination each cyme was enclosed in one of the two types of bags used in the test. Care was taken to prevent injury to the flowers.

The results of controlled pollinations obtained under field conditions in the course of controlled pollination work in 1940 indicate that, at least in this experiment, fruit set with both methods of bagging was lower than in the nonbagged controls (table 2). Kraft-paper bags yielded slightly better results than cheesecloth bags. During 1940 the environmental conditions were unfavorable for fruit set, as the temperature was relatively high and soil moisture and atmospheric humidity were low. Very few naturally pollinated fruits were formed on Katahdin plants in the same field.

The results of controlled pollinations for 1941 substantiate those obtained in 1940, in that bagging of flowers caused a substantial reduction in percentage of fruit set as compared with that in nonbagged controls (table 2). In 1941 the weather conditions from May 2 to 4 were considered favorable for fruit set, so that the difference obtained on May 2 probably represents more nearly the condition one would normally expect. The maximum temperature had become relatively high for fruit setting by May 7.

TABLE 2.—*Effect of bagging on fruit set at Baton Rouge, La., in 1940 and 1941*

CONTROLLED POLLINATIONS UNDER FIELD CONDITIONS

Isolation treatment	Date of treatment	Variety	Nature of pollination	Flowers pollinated	Flowers setting fruits	
				Number	*Number*	*Percent*
Kraft-paper bags	May 1, 1940	Katahdin	Selfing	130	3	2.3
Cheesecloth bags	do	do	do	31	0	.0
Do	do	Katahdin × (X570-84)	Cross	32	0	.0
None	do	Katahdin	Selfing	110	18	16.3
Kraft-paper bags	May 2, 1941	do	do	81	16	19.8
None	do	do	do	75	21	28.0
Kraft-paper bags	May 7, 1941	do	do	49	0	.0
None	do	do	do	37	5	13.5
REGULAR POLLINATIONS IN FEDERAL BREEDING PLOT						
Kraft-paper bags	Spring, 1940	Many	Crosses	466	79	16.9
None	do	do	do	292	58	19.8

The results of regular pollinations made in the spring of 1940 in the Federal breeding plots are also presented in table 2. These data, which represent the result of crosses of many different varieties and seedlings, tend to substantiate the previous findings that a slightly higher percentage of nonbagged flowers developed into fruit than did bagged ones.

The results of all trials uniformly show that isolation with cheesecloth or kraft-paper bags was detrimental to fruit setting under Louisiana conditions during 1940 and 1941. The cheesecloth bags were unsatisfactory in several respects. Wire frames had to be used in conjunction with them; more time was spent in applying them than paper bags; injury to essential flower parts was greater than when paper bags were used; and the fruit set was lower than when kraft-paper bags were used. On the other hand, kraft-paper bags, if used when weather conditions were generally favorable to fruit setting, gave a satisfactory percentage of successful pollination, allowing the breeder to obtain adequate seed supplies with assurance of purity.

It would appear from the literature and the results herein presented that any method of isolation of potato flowers will reduce fruit set because of their sensitivity to even slightly unfavorable environmental conditions. Bagging affects the temperature and humidity immediately surrounding pollinated flowers, probably causing more unfavorable conditions than prevail under normal field conditions.

EFFECT OF STAGE OF FLOWER DEVELOPMENT AND OF NUMBER OF POLLINATIONS ON FRUIT SET

There is no generally accepted policy among potato breeders concerning the number of pollinations necessary to give maximum fruit set or the optimum stage of flower development for such pollinations.

Stuart (*8*) recommended that flowers for controlled pollinations be emasculated 24 hours before the buds normally open. Pollen was then applied 1 or 2 days after emasculation. Uspenskii (*9*) stated that in controlled pollinations in Russia, 2 to 3 days elapse between the time of emasculation and pollination. Apparently, only one pollination is practiced. Flowers were enclosed in isolators from the

time of emasculation. Uspenskii presented evidence indicating that pollination repeated three times at different hours of the day gave a low percentage of fruit set and stated that this may be due to unnecessary irritation or injury of the stigma in the pollination process. From his results it appeared that repeated pollination decreased the percentage of fruit set when made during the afternoon and increased fruit set when made during the morning.

During 1940 data were collected to determine the effect of one and two pollinations, respectively, on fruit set. In both seasons the Katahdin variety was self-pollinated. Buds were selected that would normally have opened within 1 or 2 days. All flowers included in the test were emasculated, and the first pollination was made at that time. Those flowers given two pollinations were repollinated approximately 24 hours after emasculation and the first application of pollen. None of the cymes was bagged. In 1941 the work was broadened to include the influence on fruit setting of the stage at which flowers were pollinated. In addition to the two treatments given in 1940, some flowers were not pollinated at the time of emasculation but were given a single application of pollen about 24 hours after emasculation, or at about the time they would ordinarily be repollinated. The test was conducted over a 2-day period.

The results given in table 3 indicate that pollination at the time of emasculation was relatively ineffective in inducing fruit set. Also, the results suggest that, with two pollination treatments, beginning on the date of emasculation, the second application of pollen is far more effective than the first. Since there was apparently little or no difference in fruit setting from a single pollination made on the day following emasculation and from two pollinations, one on the day of and another on the day after emasculation, the former method seems preferable and more efficient from the standpoint of time and labor.

The two most probable reasons for poor fruit set from pollination at the time of emasculation seemed to be either that the stigma was not receptive and pollen would not germinate and penetrate the style at the time, or the pollen germinated and the tubes penetrated the style but the ovules were still immature and incapable of being fertilized. The first explanation appears logical when it is recalled that emasculation was performed while the flower was in the bud stage, about 1 or 2 days before anthesis. However, a high percentage of pollen grains placed on the stigmas of emasculated buds germinated quickly and penetrated the style in a normal manner, apparently eliminating the first hypothesis. It appears probable that the ovules are too immature to permit fertilization in the bud stage, and most of the pollen tubes from pollen applied to the stigma at this time deteriorate without effecting fertilization.

EFFECT OF SOME NATURAL ENVIRONMENTAL CONDITIONS ON FRUIT SET

References have been frequently made to the striking influence of environment on fruit setting under controlled pollination in the potato. Young (*10*) stated that degenerative changes in the ovule and embryo sac result from unfavorable environmental conditions which cause blasting of the flowers. Krantz and Hutchins (*4*) and Krantz et al. (*3*) obtained conclusive evidence that flower abscission is influenced to a considerable degree by environment.

TABLE 3.—*Effect of one and two pollinations on fruit set of selfed Katahdin potato under field conditions*

Date of first pollination	Pollinated—					
	At emasculation		24 hours after emasculation		At emasculation and 24 hours later	
	Flowers pollinated	Flowers setting fruit	Flowers pollinated	Flowers setting fruit	Flowers pollinated	Flowers setting fruit
1940	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
April 15–20	62	1.6	----------	----------	59	42.2
1941						
April 30	78	.0	87	17.2	88	1.1
May 1	88	10.3	78	25.6	75	28.0
Total or average	166	5.1	165	21.4	163	14.5

Semsroth, according to Clark (*1*), found that the best climatic conditions for the setting of fruit were a low daily mean temperature (about 65° F.) with a low maximum temperature (about 72°). A poor set resulted from a high mean daily temperature of 70° or higher, from a very low minimum and a high maximum, and from a relatively low air humidity and heavy showers on the days of emasculation and pollination.

Stevenson and Clark (*7*) stated:

Because of the effect of environment on the fruitfulness (seed production) of potato plants, it is difficult, if not impossible, for many of the State experiment stations interested in some aspect of potato-breeding work to produce true seed. * * * Certain Northern States and other States with mountainous regions where potatoes can be grown at high elevations are especially favored * * *

* * * * * * *

* * * But seed setting is also influenced by environmental factors to a marked degree. Some varieties will set seed under a wide range of conditions, while others have never been known to set seed even under favorable conditions.

* * * * * * *

* * * Conditions are favorable nearly every season at Presque Isle. A few other places in the United States have been found favorable for seed production * * *. At Estes Park, Colo., which has an elevation of 7,500 feet, seed sets in most years quite readily.

In 1940 and 1941, during the course of the regular breeding work at Baton Rouge, La., records were kept of the date of pollination, number of flowers pollinated, and the number of fruit set. Compilation of these data according to date of pollination brought out some interesting relationships between environment and fruit setting in the potato. The data for the 2 years are presented in table 4.

From the data in table 4, it is apparent that in 1940 the percentage of flowers that set fruits was progressively less each day after pollination work was started on May 2. A record of weather conditions at Baton Rouge for this period indicates that the mean temperature varied markedly during the pollination period, May 2 to May 15. The mean temperature during the 10 days preceding May 2 was relatively low, and the minimum temperature for May, 51° F., was recorded on May 2. Beginning with May 4, the mean temperature rose gradually until the highest temperature for the month, 90°, was recorded on May 16. The weather report for the period shows 2.5

inches of rainfall during the 10 days previous to May 2, which was the first and most successful date of pollination. This provided high soil moisture and atmospheric humidity for development of the flower buds during the first 2 or 3 days of pollination. On each date after May 2 soil moisture and atmospheric humidity became noticeably lower. Rising daily temperatures and declining soil moisture characterized the period from May 4 to May 15 and probably were the principal causes of the low fruit set after May 4. Very few naturally pollinated fruits developed on normally highly fertile varieties during that period; this also indicates the influence of unfavorable weather conditions. Thus, it seems that a combination of declining soil moisture and humidity and rising daily temperatures was probably the most influential factor in the daily decrease in percentage of flowers that set fruits from May 2 to May 8.

TABLE 4.—*Fruit set on different dates of pollination during the regular pollination work, Baton Rouge, La., in 1940 and 1941*

Date of pollination	Flowers pollinated	Flowers setting fruit	Date of pollination	Flowers pollinated	Flowers setting fruit
1940	*Number*	*Percent*	*1941*—Continued		
May 2	141	46.0		*Number*	*Percent*
3	113	26.5	April 21	114	20.2
4	103	20.4	22	167	23.4
6	116	9.5	23	84	17.9
7	118	6.8	25	267	12.5
8	40	5.0	26	82	15.8
10	11	0	28	125	28.8
11	56	0	29	10	20.0
15	49	0	30	139	7.2
			May 1	132	15.9
1941			4	42	19.0
April 18	40	12.5	7	65	15.4
19	105	17.1	8	93	23.7
20	127	21.3	9	90	25.6

The results for the 1941 season (table 4) do not show the adverse influence of environment which characterized the previous season. In 1941 conditions were generally more favorable for fruit setting, since no extreme fluctuations in temperature and soil moisture occurred. Furthermore, rainfall was well distributed over the period, being recorded as follows: 0.10 inch, April 25; 0.13 inch, April 26; 0.60 inch, April 29; 1.64 inches, April 30; and 0.31 inch, May 1. However, no rain fell after May 1 until May 24; the total precipitation for the month was 4.62 inches below normal. Most of the variations in fruiting during 1941 can be accounted for by differences in male and female parents used on the various dates of pollination.

As the temperature is generally high during May in Baton Rouge, it seems advisable to plant parental breeding material in the field early enough for pollination work to start by April 15 to 20. The results obtained on fruit set during the latter part of April and early May in 1941 indicate that ordinarily no trouble should be encountered in securing adequate seed supplies in Louisiana.

DISCUSSION AND SUMMARY

Results of the investigations on the prevalence of nonfruitfulness in potato varieties due to pollen sterility and premature abscission of flowers generally substantiate the reports of other workers in different

regions. However, the data do show that an encouraging number of fruitful varieties and seedling varieties possessing superior germ plasm are available to the potato breeder. Records on fruitfulness of varieties should have practical application in enabling the breeder to make more efficient use of the available material.

Isolation of potato flowers to prevent contamination with foreign pollen by enclosing the flower clusters in kraft-paper or cheesecloth bags was detrimental to fruit setting in Louisiana during the 1940 and 1941 seasons. The reduction in fruit set from the use of bags was apparently due in the main to alteration of temperature and humidity relations around the flowers. Of the two types of bags used, kraft-paper bags proved most satisfactory from the standpoint of time required in application. The results indicate that under reasonably favorable weather conditions adequate supplies of seed can be secured under kraft-paper bags in Louisiana.

Application of pollen at the time of emasculation of flowers (in the bud stage) was relatively ineffective in inducing fruit set in the Katahdin variety. Results with only one pollination 24 hours after emasculation indicate that this method was as efficacious as pollination simultaneously with emasculation, followed by a second pollination 24 hours later. In view of the results secured in this study, it would seem advisable to emasculate and bag potato flowers one day and apply pollen on the following day.

High temperature and low moisture or low moisture proved unfavorable to fruit setting in the potato. The results reported are in close accord with the findings of other investigators and demonstrate the marked sensitivity of fruiting in the potato to the environment. Environmental conditions favorable to general growth are apparently favorable to fruiting. Thus, it is advisable that, in conducting a potato-improvement program by breeding, the breeder should arrange to have the pollination work come during a period in which relatively low temperature and adequate soil moisture conditions normally prevail.

Natural cross-pollination in potatoes, although not investigated in the work herein reported, is undoubtedly possible, and it may occur to a small degree in unbagged flowers under field conditions in Louisiana. The extent of cross-pollination cannot be stated for any region with any degree of precision. The potato breeder should recognize this possibility of contamination with foreign pollen in conducting pollination work, and each worker must make the decision as to whether bagging of flowers, with consequent loss of time and labor and lower percentage of fruiting, is necessary or advisable to his program. It may be argued that the degree of crossing is so low as to be negligible in the ordinary breeding program, especially where no genetic interpretation is to be placed on the results. Nevertheless, in obtaining seed for use in genetic studies, isolation of flowers by some method of bagging would seem advisable.

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ISOLATION OF CERATOSTOMELLA ULMI FROM INSECTS ATTRACTED TO FELLED ELM TREES [1]

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INTRODUCTION

The role of insects in the dissemination of *Ceratostomella ulmi* Buisman, the fungus that causes the Dutch elm disease, seems to be twofold. The insect contaminated with the fungus may feed directly on a living elm tree and thereby cause infection, or it may establish *C. ulmi* in dead or dying elm wood, whence its progeny or other insects may carry the fungus when they emerge. A knowledge of what insects are involved, and of their relative importance, is an essential part of the foundation upon which measures for the eradication and control of the disease must be based.

During the years 1936–39 insects were collected from recently felled elm trees at selected locations in New Jersey and New York. For the most part these insects were species known to breed in elms. They were later cultured to determine what species were carrying *Ceratostomella ulmi*, and to find the percentage of individuals of each species that were contaminated with the fungus. The results of this investigation are presented in this paper.

METHODS OF COLLECTING AND CULTURING INSECTS

The insects were taken from the surface of the bark of healthy American elms (*Ulmus americana* L.) that had been felled and placed horizontally on supports about 2 feet from the ground. They were collected individually in new gelatin capsules, care being taken to prevent contamination. The insects were sent by mail daily to the Morristown, N. J., laboratory, where they were identified without being removed from the capsules and refrigerated at 5° C. or lower temperatures until they were cultured.

The insects were cultured individually according to the following method, a modification of that described by Walter.[4] A Petri dish containing two pieces of filter paper and a chip from a small twig of seasoned elm was autoclaved for 20 minutes at 15 pounds' pressure and moistened with 4 cc. of sterile distilled water. The insect was then crushed on the chip with a sterile forceps, and the Petri dish was

[1] Received for publication February 25, 1942. The investigation reported was a cooperative one between the Dutch Elm Disease Eradication unit and the Division of Forest Insect Investigations of the Bureau of Entomology and Plant Quarantine, and the Division of Forest Pathology of the Bureau of Plant Industry. Members of the Dutch Elm Disease Eradication unit cut, placed, and later removed the elm trees from which the insects were collected, and employed men to collect and culture these insects.

[2] Died Feb. 22, 1941.

[3] The writers wish to acknowledge their indebtedness to those members of the three cooperating agencies who assisted in this work in various ways, especially the following members of the Bureau of Entomology and Plant Quarantine: To C. H. Hoffmann for determining many of the insects, to D. O. Wolfenbarger for assistance in supervising the field work in 1936, to R. R. Whitten for advice regarding the sampling method used in 1939, and to A. E. Lantz for preparing the figures that accompany this article.

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incubated for about 20 days at 10° to 15° C. Since the typical fruiting structure of the imperfect stage of *Ceratostomella ulmi* is a coremium about 1,200 to 1,500μ in height, it is fairly conspicuous, especially when observed under a binocular dissecting microscope. Transfers were made from the coremial heads to malt or potato-sucrose agar plates with a sterile needle. Identification of *C. ulmi* was made by microscopic examination of the colonies produced by these transfers. This procedure was necessary because coremia-forming fungi other than *C. ulmi* were sometimes present in the cultures.

INVESTIGATIONS IN 1936

In 1936 three felled trees were installed on May 18 in sunny situa-

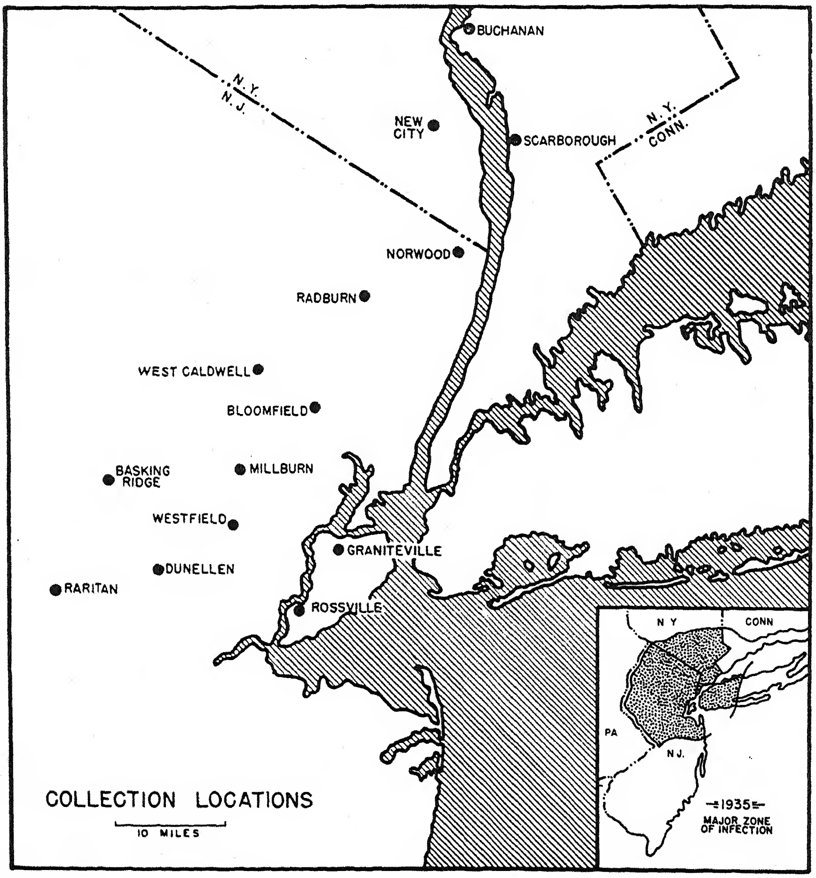

FIGURE 1.—Locations in New Jersey and New York where insects were collected. Insert shows major zone of Dutch elm disease infection as known in December 1935 and within which the insect collections were made.

tions at each of six places in New Jersey where elm trees affected by the Dutch elm disease had been removed previously in some numbers. These locations were in Basking Ridge, Westfield, Bloomfield, Radburn, Millburn, and West Caldwell (fig. 1). After 2 weeks one of the trees at each location was removed, burned, and replaced by a newly felled tree. This procedure was repeated every 2 weeks so that three

felled trees were exposed at all times at each location and no tree was left longer than 6 weeks.

A man was assigned to each location with instructions to collect all adults seen on the bark of the trees. It might be expected that numerous individuals of species having no intimate connection with elm would be taken, but as a matter of fact most of the insects taken were of species known or suspected to develop in elm, either by feeding in the wood and bark or as parasites and predators on insects breeding in elm.

The collectors worked at all locations from May 25 to September 9, and some of them for a few days before or after this period. Each man was on duty on week days from 8 a. m. to 4:30 p. m. (eastern daylight-saving time) and on Saturdays from 8 a. m. to noon, except that once each week his hours were from 4 p. m. to midnight. At night the men used electric lanterns to aid them in finding insects on the trap trees, but no trapping devices other than the felled trees were employed.

TABLE 1.—*Isolation of Ceratostomella ulmi from insects collected at six selected locations in New Jersey in 1936*

Insect	Insects cultured	Insects from which *C. ulmi* was isolated
Coleoptera:		
Scolytidae:	*Number*	*Percent*
Scolytus multistriatus (Marsh.)	7, 209	6. 9
Scolytus sulcatus Lec.	1	0
Hylurgopinus rufipes (Eich.)	139	4. 3
Xylosandrus germanus Bldfd.	826	. 2
Xyleborus sp.	25	0
Chramesus hicoriae Lec.	1	0
Other Scolytidae	1	0
Bostrichidae: *Xylobiops basilaris* (Say)	114	1. 8
Cerambycidae:		
Saperda tridentata Oliv	78	0
Neoclytus acuminatus (F.)	555	0
Xylotrechus colonus (F.)	89	0
Other Cerambycidae	2	0
Buprestidae:		
Chrysobothris femorata (Oliv.)	202	0
Anthaxia viridifrons Gory	83	0
Other Buprestidae	2	0
Curculionidae:		
Magdalis armicollis (Say)	1, 419	. 1
Magdalis barbita (Say)	338	0
Magdalis inconspicua Horn	474	0
Conotrachelus anaglypticus (Say)	143	. 7
Other Curculionidae	2	0
Cleridae:		
Enoclerus quadriguttatus (Oliv.)	9	0
Chariessa pilosa (Forst.)	15	0
Other Cleridae	1	0
Histeridae:		
Platysoma coarctatum Lec.	3	0
Other Histeridae	2	0
Lampyridae	27	0
Nitidulidae: *Colopterus semitectus* (Say)	1	0
Corylophidae: *Molamba* sp.	1	0
Melandryidae: *Synchroa punctata* Newm.	9	0
Elateridae	37	0
Tenebrionidae	1	0
Chrysomelidae: *Microrhopala vittata* (F.)	4	0
Hymenoptera:		
Xiphydriidae: *Xiphydria* sp.	2	0
Braconidae: *Capitonius saperdae* (Ashm.)	5	0
Formicidae	58	0
Hemiptera:		
Membracidae:		
Stictocephala lutea (Walk.)	65	0
Other Membracidae	3	0
Pentatomidae: *Brochymena* sp.	1	0
Total	11, 947	

The insects collected and cultured in 1936, as well as the percentages yielding *Ceratostomella ulmi*, are given in table 1. The species showing the highest percentage of infection were *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eich.), and the fungus was isolated from four other species. The numbers of *S. multistriatus* and *H. rufipes* collected at each of the six locations, and the percentages from which *C. ulmi* was obtained, are shown in table 2.

Figure 2 shows graphically how the numbers of *Scolytus multistriatus* taken during weekly collection periods at all locations, and the percentages of these beetles found to be contaminated with *Ceratostomella ulmi*, varied throughout the season. Only five beetles were collected during the May 24–31 and the September 13–19 periods in 1936, and it is on these limited numbers that the indicated 20 percent of contaminated beetles is based. The small number of *S. multistriatus* adults taken between July 12 and 25 is interpreted as being due to the fact that this period fell between the peaks of abundance of adults of the first and second generations.

The number of collected adults of the various species known to breed in elm cannot be used as a basis for estimating the comparative abundance of these species, even in the immediate vicinity of the trap trees. It is certain, for instance, that had the trees been installed earlier in 1936 and in the subsequent years in which the investigation was conducted, more *Hylurgopinus rufipes* would have been collected. Sixty percent of the insects collected were *Scolytus multistriatus*. This species is recognized as the most important insect vector of the Dutch elm disease fungus in this country.

Since the sex of *Scolytus multistriatus* adults can be determined readily, it was deemed advisable to ascertain whether a greater percentage of one sex than of the other was carrying the fungus. The sex was determined before the beetles were cultured. Males composed 71.2 percent of the total number, no doubt because the males run about more on the surface of the bark than do the females and were thus more often seen by the collectors. *Ceratostomella ulmi* was isolated from 6.9 percent of the males and from 7.1 percent of the females.

TABLE 2.—*Isolation of Ceratostomella ulmi from Scolytus multistriatus and Hylurgopinus rufipes collected at selected locations in 1936, 1937, 1938, and 1939*

Location	From *Scolytus multistriatus*								From *Hylurgopinus rufipes*							
	Insects collected in—				Insects giving *C. ulmi* in—				Insects collected in—				Insects giving *C. ulmi* in—			
	1936	1937	1938	1939	1936	1937	1938	1939	1936	1937	1938	1939	1936	1937	1938	1939
	Number	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
New Jersey:																
Basking Ridge	620	2,685	1,960	1,400	8.5	6.8	11.3	6.75±1.55	72	566	84	356	4.2	2.6	1.2	0.8
Westfield	1,551	1,625	8,940	9,357	6.9	5.4	12.7	1.75±0.66	0	1	144	107	0	0	4.2	0
Bloomfield	2,461	3,925	2,162	5,143	4.9	5.7	3.9	6.00±1.28	1	5	6	7	100.0	0	0	14.3
Radburn	497	542	5,281	1,020	3.0	.9	2.3	3.00±1.33	0	0	0	1	0	0	0	0
Millburn	1,414	972	6,339	3,517	9.5	5.7	8.7	9.25±1.41	11	6	15	51	0	0	0	0
West Caldwell	666	47	8,510	6,167	10.1	19.2	5.1	7.50±0.92	55	34	25	32	3.6	0	8.0	0
Total or average	7,209	9,796	33,192	26,604	6.9	5.8	7.7	5.71±2.51	139	612	274	554	4.3	2.4	3.3	.7
Norwood		410				2.9				0				0		
Raritan		2,321				5.1				43				2.3		
Dunellen		380				3.9				0				0		
New York:																
New City		280				3.2				63				1.6		
Scarborough		1,218				2.3				122				.8		
Buchanan		827				.8				65				0		
Rossville		351				.8				0				0		
Graniteville		2				0				0				0		
Grand total or average		15,585				4.8				905				2.0		

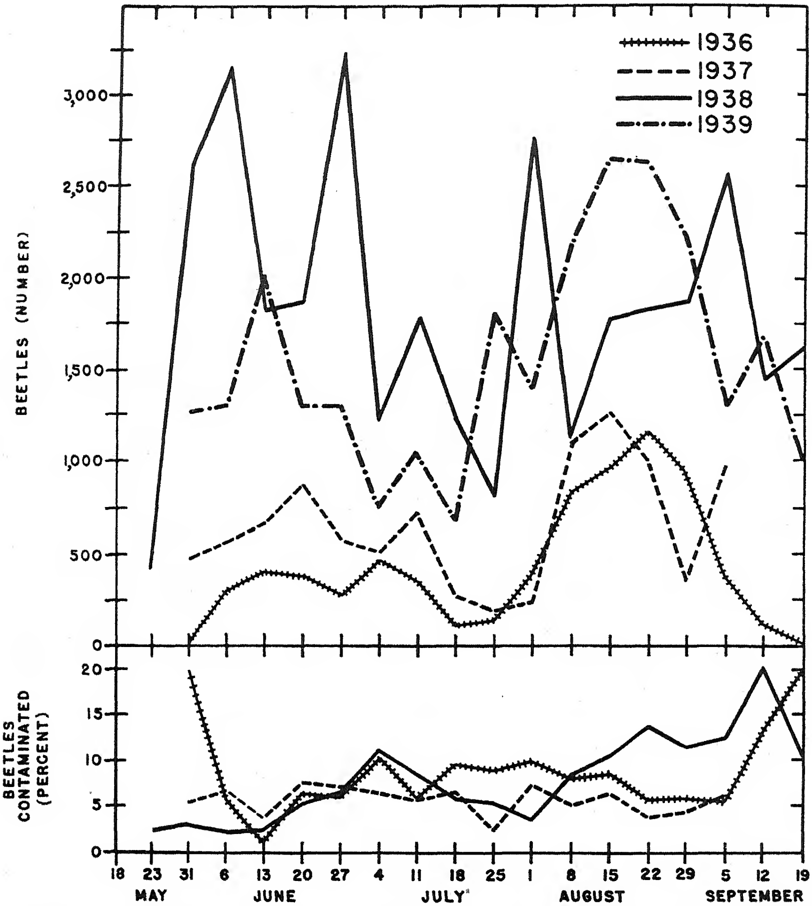

FIGURE 2.—Numbers of *Scolytus multistriatus* adults collected during the seasons of 1936–39, and the percentages of these beetles contaminated with *Ceratostomella ulmi* during the first 3 years. Collections from six locations in New Jersey have been combined for the periods indicated.

INVESTIGATIONS IN 1937

Freshly cut elm trees were installed on May 17, 1937, at the same places in New Jersey in which insect collections had been made during 1936. Trees were also installed at eight additional places—Norwood, Raritan, and Dunellen, N. J., New City, Scarborough, and Buchanan, N. Y., and Rossville and Graniteville on Staten Island, N. Y. (fig. 1). Four trees were placed at each location, three in a sunny situation and the fourth where it would be shaded most of the time. The trees in the sunny situation were later removed and replacements made as in 1936. The tree in the shady location was removed every 4 weeks and another put in its place.

Since in 1936 *Ceratostomella ulmi* was isolated more frequently from *Scolytus multistriatus* and *Hylurgopinus rufipes* than from the other species, and since these species are considered to be the most important

insect carriers of *C. ulmi* in the United States,[5] the collections of 1937 were limited to these two species.

The collectors began work on May 17, but they captured no bark beetles until May 24. At the six sites used in 1936 collecting was continued until September 5, but at the eight additional sites it was discontinued on July 14. The men were at each location on alternate days only, instead of daily as in 1936.

No night collecting was done in 1937. The men worked from 9 a. m. until 6 p. m., for it had been found in 1936 that 8 a. m. was early for insect activity and that activity continued after 4:30 p. m. Therefore, they probably captured more insects during any one day than had the hours been the same as in 1936. Because of this change in hours, because collections were made only on alternate days at any one place in 1937, and for other reasons the difference in numbers of *Scolytus multistriatus* or *Hylurgopinus rufipes* taken during the 2 years does not represent yearly fluctuations in abundance of either species.

The methods of identifying and culturing the insects in 1937 were similar to those in the previous year. The numbers of *Scolytus multistriatus* and *Hylurgopinus rufipes* taken at each location and the percentages of each yielding *Ceratostomella ulmi* are shown in table 2. As in 1936, *C. ulmi* was isolated from a higher percentage of *S. multistriatus* than of *H. rufipes*. The combined number of *S. multistriatus* taken at the six locations during approximately weekly periods and the percentages of these beetles from which *C. ulmi* was isolated are indicated in figure 2.

The sex ratio of *Scolytus multistriatus* approached that in 1936. In 1937, 76.6 percent of this species were males. *Ceratostomella ulmi* was isolated from 6.0 percent of the males and from 5.0 percent of the females.

INVESTIGATIONS IN 1938

In 1938 four trap trees were again put in place at each of the six sites used in 1936 and 1937. Three trees were again placed in a sunny situation and one in a shaded position. The schedule for removing the trees and replacing them by others was the same as in 1937. Collectors were at each location on alternate days from May 16 to September 17. Their hours were the same as in 1937, and they again collected only *Scolytus multistriatus* and *Hylurgopinus rufipes*.

Table 2 includes the numbers of the two species taken at each of the six locations, and the percentages of each from which *Ceratostomella ulmi* was obtained. Figure 2 indicates the combined number of *Scolytus multistriatus* taken at the six sites during intervals of approximately a week throughout the collecting season, and the percentages of these beetles from which *C. ulmi* was cultured. No separation of the sexes of *S. multistriatus* was made in 1938.

From figure 2 it will be noted that many more *Scolytus multistriatus* were collected in 1938 than in either of the 2 previous years, particularly in the early part of the season. The marked increase is believed to be due to the fact that numerous elms near four of the sites had been treated with dry copper sulfate late in 1936 or early in 1937. These elms were of small value, and the Dutch Elm Disease Eradica-

[5] MAY, C., and COLLINS, C. W. THE DUTCH ELM DISEASE IN THE UNITED STATES AND ITS INSECT VECTORS. West. Shade Tree Conf. Proc. 5: 49–54. 1938.

tion unit treated them with the chemical for the purpose of killing them and thus reducing the number to be inspected for external symptoms of the disease.[6] When the work was done it was not expected that bark beetles would attack these chemically treated trees. They did, however, and many of the *S. multistriatus* adults that were taken from the trap trees in 1938 may have originated in these trees. Conversely, it is also probable that the treated trees influenced the number of *S. multistriatus* collected from the trap trees in 1937, because the trees treated with copper sulfate were more attractive to the beetles than were the trap trees.

Figure 2 also indicates that a larger percentage of *Scolytus multistriatus* beetles collected during the latter part of 1938 were contaminated with *Ceratostomella ulmi* than of the beetles collected earlier. Most of these contaminated beetles were taken from one location, where the collections were comparatively large.

INVESTIGATIONS IN 1939

The procedure followed in installing the trap trees and collecting insects in 1939 was the same as that used in 1938. The collectors reported for duty on May 22 and worked until September 16.

It was impossible to culture all the *Scolytus multistriatus* beetles collected in 1939. Consequently, the capsules containing those beetles taken at each location were thoroughly mixed, and 20 samples of 20 beetles each were drawn at random and then cultured in the usual manner.[7] The culture results were analyzed statistically according to standard methods. Table 2 indicates the number collected at each location and the percentages from which the fungus was obtained.

Figure 2 shows the total number of *Scolytus multistriatus* taken at the six sites at intervals of approximately a week. Since the collections for the entire season were combined, it is impossible to show the weekly variation in the percentages of beetles carrying the fungus, as has been done for previous years.

All *Hylurgopinus rufipes* adults collected in 1939 were cultured. The number taken at each location and the percentages of each from which the fungus was obtained are given in table 2.

SUMMARY

Experiments were conducted from 1936 to 1939 to ascertain what species of insects were carrying the Dutch elm disease fungus (*Ceratostomella ulmi* Buisman) and from what percentage of each of these species the organism could be isolated. Insects attracted to felled healthy elm trees placed at several locations in New Jersey and New York were collected, identified, and cultured.

In 1936 all adult insects found on the surface of the bark of felled elms at six sites in New Jersey were collected and cultured. They included identified individuals of 23 species and many other specimens that were determined only to family or genus. *Ceratostomella ulmi* was isolated most frequently from two species of elm bark beetles, *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eich.). It was also isolated from four other species of Coleoptera, namely,

[6] BREWER, E. G., and MIDDLETON, W. DUTCH ELM DISEASE ERADICATION; JAPANESE BEETLE CONTROL; EUROPEAN CORN BORER AND GYPSY MOTH CERTIFICATION. Jour. Econ. Ent. 31: 577-583. 1938.

[7] In 1939, all the culture work was done under the supervision of L. M. Fenner and E. G. Kelsheimer, of the Bureau of Entomology and Plant Quarantine.

Xylobiops basilaris (Say), *Conotrachelus anaglypticus* (Say), *Xylosandrus germanus* Bldfd., and *Magdalis armicollis* (Say).

Because of the results of the 1936 experiment, and because *Scolytus multistriatus* and *Hylurgopinus rufipes* are considered the most important insect vectors of *Ceratostomella ulmi* in the United States, only adults of these two species were cultured in 1937, 1938, and 1939. They were collected at the same six locations as in 1936 Many more *S. multistriatus* than *H. rufipes* were taken. *C. ulmi* was obtained from 6.9, 5.8, 7.7, and 5.71 percent of the *S. multistriatus*, and from 4.3, 2.4, 3.3, and 0.7 percent of the *H. rufipes*, cultured in 1936, 1937, 1938, and 1939, respectively. The percentage of beetles contaminated with *Ceratostomella ulmi* varied considerably at different locations in the same year and at the same location in different years. In 1937 *Scolytus multistriatus* and *H. rufipes* collected at eight additional locations in New Jersey and New York were cultured.

In 1936 and 1937 the sex of the *Scolytus multistriatus* adults was determined before they were cultured. Males composed 71.2 percent of the adults in 1936 and 76.6 percent in 1937. In 1936 *Ceratostomella ulmi* was cultured from 6.9 percent of the males and from 7.1 percent of the females; in 1937 the percentages were 6.0 and 5.0, respectively.

DEPOSITS OF INSECTICIDAL DUSTS AND DILUENTS ON CHARGED PLATES [1]

By G. F. MacLeod, *entomologist*, and Leslie M. Smith, *assistant entomologist, Division of Entomology and Parasitology, California Agricultural Experiment Station*[2]

INTRODUCTION

Preliminary tests on avocado leaves directed attention to the importance of the relation of frictional charges on dust particles to the deposition and adhesion of insecticides. A review of the literature showed that the physical influence of diluents upon the insecticidal action of the active principle in dust mixtures had not received the attention that it merits.

The possible interrelations of some physical factors that affect the insecticidal action of a dust are set forth in the flow chart shown in figure 1. Aside from chemical toxicity, the insecticidal action of a dust will depend primarily upon deposition, i. e., putting the powder on the plant or insect; and adhesion, i. e., the ability of the powder to remain in place under weathering influences. Deposition may be dependent upon elutriation, i. e., separation by differential flotation, and upon frictional charge, i. e., an accumulation of static charges caused by the dust striking against other materials while being blown to the plants. Elutriation is obviously controlled by particle size and particle density, provided complete deflocculation is assumed. Since, however, in commercial dusting practice many aggregates are present in the dust cloud, those factors that contribute to the formation of aggregations are indirect factors influencing elutriation. Some such factors are moisture content, frictional charge, particle size, and cohesion. Frictional charge may be influenced by the moisture content of the dust, by climatic conditions, by the dusting machine, and, in the case of a mixture of two or more powders, by friction between unlike particles. Some of the factors which influence the development of a frictional charge on insecticidal powders as well as some of the possible effects of such a charge on the behavior of the dust are given in the flow chart in figure 2.

EQUIPMENT AND METHODS

An attempt has been made to measure the effect of several factors on deposition, adhesion, elutriation, and formation of aggregations. For this purpose, an apparatus was devised (fig. 3) consisting of a Niagara rotary hand duster, a copper-lined sedimentation chamber, and two copper plates which, when charged, maintained an electrostatic field between them. The feeder opening on the duster was calibrated in

[1] Received for publication June 4, 1942. Assistance was rendered by the Work Projects Administration through Project O. P. 465-03-3-168.

[2] The authors gratefully acknowledge the patient and careful criticisms of this work received from Dr. Leonard B. Loeb, professor of physics at the University of California.

eighths of the total area. Metal outlet tubing was either the standard Niagara galvanized-iron tubing or galvanized-iron drain pipe. The rubber tubing used was radiator hose, garden hose, and compressed-air coupling hose such as are used on freight cars.

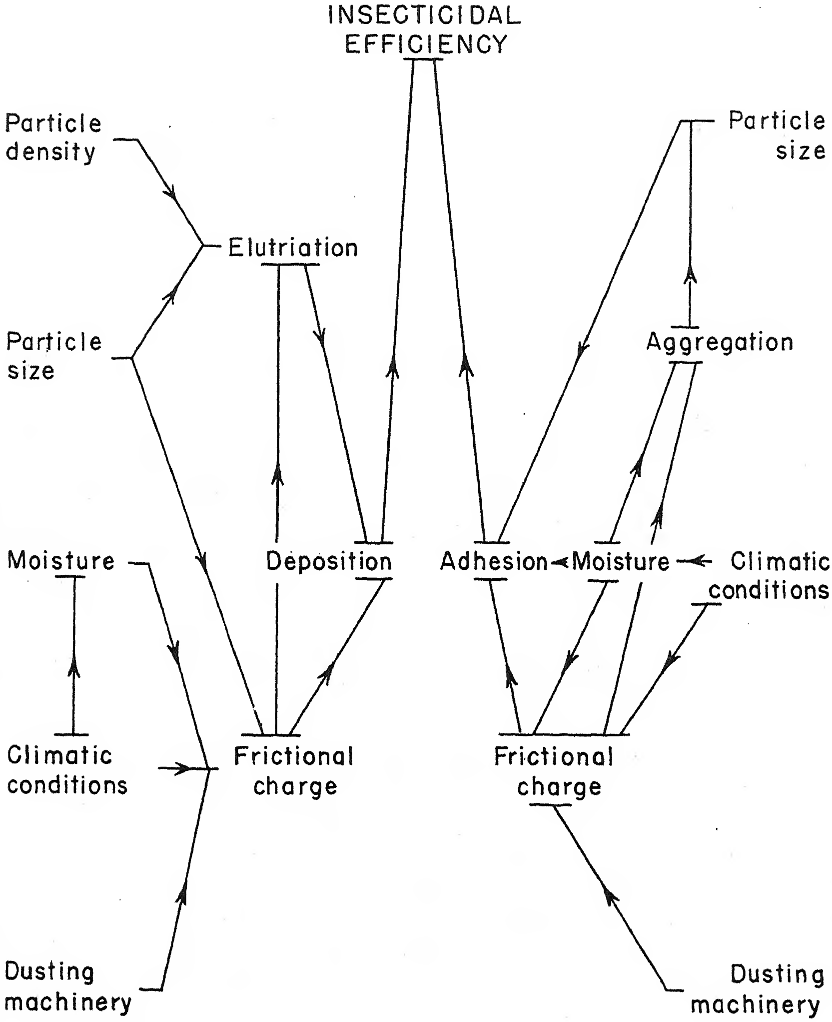

FIGURE 1.—Flow chart showing possible interrelations of some physical factors influencing the insecticidal action of the active ingredient in a dust mixture.

The sedimentation chamber, 50 inches long, provided a space in which the larger particles, lumps, and aggregations dropped out of the air stream. If these large masses were blown across the charged plates they swept off the deposit of charged material, thereby introducing an error.

The charged plates, 12 inches square, were shellacked on their inner faces. They were symmetrically arranged on a vertical plane, ½ inch

apart at the top and ¾ inch apart at the bottom, so that no dust could fall onto them from above or be held there by gravity. The usual working potential on the plates was 500 volts, direct current (supplied from a rectifying tube on 110-volt alternating current with a fluctuation of about 2 volts. For certain studies the voltage was controlled between 200 and 500 volts by means of two variable resistances.

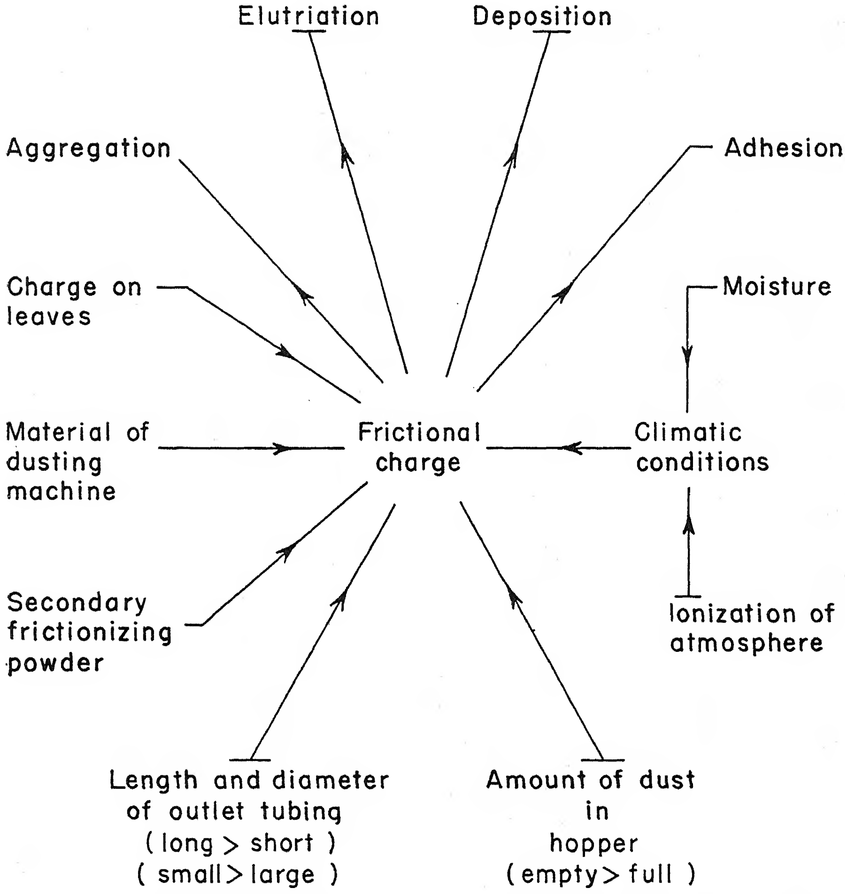

FIGURE 2.—Flow chart showing some of the factors which influence development of a frictional charge on insecticidal powders as well as some of the possible effects of such a charge on the behavior of the dust.

In operation, the crank of the duster was turned by hand at a relatively uniform, slow speed until a significant weight of dust was judged to have adhered to the plates. The hinged plates were then opened downward like a book, and the deposit on each plate removed over the area covered by a single swath of a 10-inch rubber squeegee. The dust removed from each plate was weighed.

In all cases care was taken by means of adequate control experiments to make sure that the deposit of dust was never too thick to interfere with proper deposition. The allowable density of deposit

for proper deposition varied with various dust samples and all data were taken within the allowable limits. To eliminate any effects of asymmetry in the apparatus or air stream measurements of successive runs were made by reversing the polarity of the plates and averaging the two runs.

EXPERIMENTAL RESULTS AND DISCUSSION

Since a large portion of any dust is not deposited on the plates but is blown out beyond, the question arose as to how this material would be deposited on charged plates. To answer this question, the apparatus was equipped with electrodes 12 inches high and 4 feet long. These longer plates collected practically all of the dust. Comparable and opposite areas 12 inches wide were laid off along these plates, and the percentage of material deposited on each plate was determined as shown in table 1. The major deposit was computed as per-

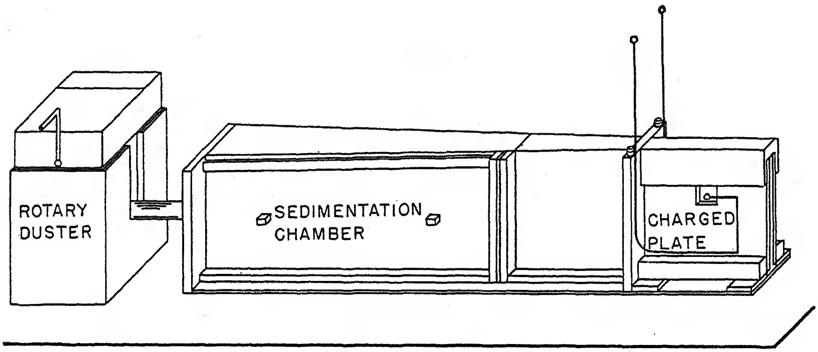

FIGURE 3.—Apparatus devised for measuring the effect of several factors on the deposition, adhesion, elutriation, and formation of aggregations in insecticidal dusts.

cent of the total deposit on both plates. The weighted averages were computed by the formula $\frac{T'P'+T''P''+\text{etc.}}{\Sigma T}$, wherein T is the total weight of dust collected at a given mean distance along the plates and P is the percentage of the major deposit composing that weight of dust.

TABLE 1.—*Deposits of dusts at various points on 4-foot electrodes*

Powder	Mean distance along plates	Total weight of dust collected	Major deposit	Weighted averages major deposit
	Inches	*Milligrams*	*Percent*	*Percent*
Soapstone No. 1	10	595	77.0	73.3
	25	364	72.1	
	39	302	67.5	
Rotenone dust No. 1	10	100	75.9	72.7
	25	147	70.0	
	39	120	70.5	

In all the data hereafter presented, the ratio of material (heavier deposit: total deposit) has been determined on electrodes 12 inches square placed at a distance comparable to the 10-inch mean distance

indicated in table 1, and for relative conclusions these data probably suffice. The true ratio for all the dust blown from the duster, however, may be somewhat lower (table 1).

The deposits which were obtained when several insecticidal powders and common diluents were blown through the apparatus described above with 20 inches of galvanized-iron outlet tubing on the duster, are given in table 2.

TABLE 2.—*Ratios of dust deposit when 20 inches of galvanized-iron outlet tubing was used on the duster*

Material	Charge on plate	Proportion of total deposit	Material	Charge on plate	Proportion of total deposit
		Percent			*Percent*
Redwood flour No. 1	Negative	93.6	Magnesium carbonate No. 1	Negative	57.8
Lead arsenate No. 2	do	89.2	Sulfur No. 10	do	57.6
Calcium arsenate No. 1	do	86.3	Sulfur No. 12	do	50.8
Calcium carbonate No. 1	do	86.2	Sulfur No. 2	Positive	51.6
Lead arsenate No. 1	do	80.6	Clay No. 2	do	56.9
Rotenone powder No. 1	do	80.3	Cryolite No. 1	do	58.5
Pyrethrum No. 1	do	80.0	Sulfur No. 14	do	60.7
Phenothiazine No. 1	do	76.9	Sodium fluosilicate No. 1	do	61.4
Dextrin No. 1	do	73.8	Soapstone No. 1	do	68.5
Calcium hydroxide No. 1	do	72.9	Talc No. 1	do	71.2
Calcium carbonate No. 3	do	71.2	Copper cyanide No. 2	do	79.9
Walnut-shell powder No. 2	do	70.4	Diatomite No. 2	do	80.5
Magnesium oxide No. 1	do	70.0	Diatomite No. 1	do	81.2
Rotenone powder No. 2	do	68.5	Sulfur No. 3	do	81.3
Barium fluosilicate No. 1	do	67.0	Calcium carbonate No. 2	do	81.5
Redwood bark No. 1	do	64.8	Clay No. 1	do	83.6
Sulfur No. 1	do	61.2	Bentonite clay No. 2	do	92.3
Gypsum No. 1	do	59.2	Bentonite clay No. 1	do	93.4

All the powders listed in table 2 were used in the form supplied to the insecticide trade; e. g., rotenone dusts were undiluted cube and derris powders, and the pyrethrum, fluosilicate, arsenate, etc., powders were also used undiluted. The several sulfur dusts were commercial brands which contained so-called conditioners.

The ratio of the deposits varied widely with variations in the dusting apparatus, with variations in the dust, and possibly with variations in the atmosphere. Several powders were blown through the sedimentation chamber and then between the charged plates by holding a pile of dust on a large spatula in front of the air blast from the rotary duster at the entrance to the sedimentation chamber. These dusts did not come in contact with the duster or the outlet tubing. The results are given in table 3.

TABLE 3.—*Deposits occurring when dust was blown from in front of outlet tube*

Material	Charge on plate	Proportion of total deposit	Gain or loss over deposit of dust blown through metal outlet tube (table 2)
		Percent	*Percent*
Calcium arsenate No. 1	Negative	78.0	−8.3
Barium fluosilicate No. 1	do	65.4	−1.6
Bentonite clay No. 1	Positive	90.3	−3.1
Diatomite No. 2	do	70.5	−10.0
Redwood flour No. 1	Negative	69.3	−24.3
Rotenone powder No. 1	do	68.4	−11.9
Soapstone No. 1	Positive	74.6	+6.1
Walnut-shell powder No. 2	Negative	80.1	+9.7

A 20-inch tube of brown wrapping paper was substituted on the duster for the 20-inch iron tube used in the first tests reported in table 2. Several powders were blown through such paper tubing with the results shown in table 4.

TABLE 4.—*Deposit occurring when 20 inches of paper outlet tubing was used*

Material	Charge on plate	Proportion of total deposit	Gain or loss over deposit of dust blown through metal outlet tube (table 2)
		Percent	*Percent*
Rotenone powder No. 2	Negative	63.2	−5.3
Rotenone powder No. 1	do	65.0	−15.3
Bentonite clay No. 1	Positive	90.9	−2.5
Redwood bark No. 1	do	69.8	+34.6
Redwood flour No. 1	do	52.4	+90.0
Cryolite No. 1	do	81.8	+23.3

The influence of the length of the outlet tubing on the ratio of deposits was tested by blowing several powders through iron or paper tubes of various lengths attached to the duster. The results are given in table 5 and shown graphically in figure 4.

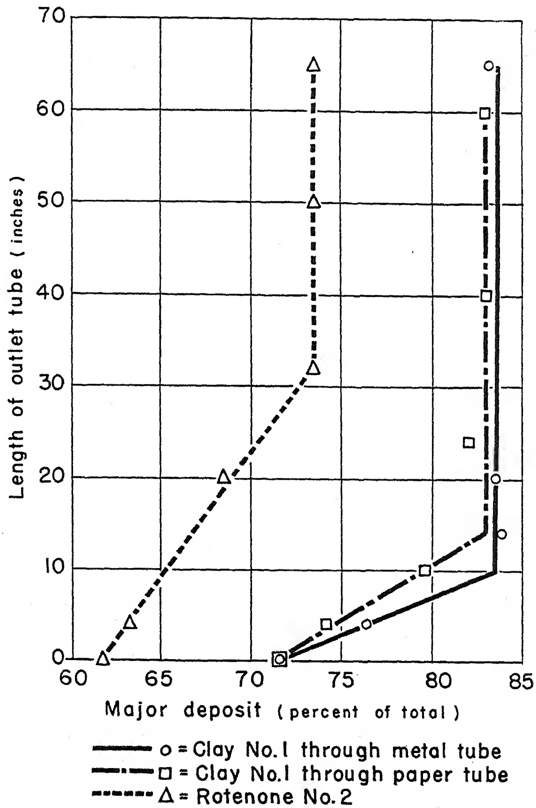

FIGURE 4.—The influence of the length of the outlet tubing on the ratio of dust deposits.

TABLE 5.—*Relation of the length of outlet tubing to ratio of deposits of dusts on electrodes*

METAL TUBING

Material	Tube length	Charge on plate	Proportion of total deposit
	Inches		*Percent*
Clay No. 1	0	Positive	71.6
	4	do	76.3
	14	do	83.9
	20	do	83.6
	65	do	83.2
Rotenone powder No. 2	0	Negative	61.8
	4	do	63.4
	20	do	68.5
	32	do	73.4
	50	do	73.5
	65	do	73.4
Talc No. 2	0	Positive	77.0
	20	do	80.0
	60	do	83.4

PAPER TUBING

Material	Tube length	Charge on plate	Proportion of total deposit
Clay No. 1	0	Positive	71.6
	4	do	74.2
	10	do	79.6
	24	do	82.0
	40	do	83.0
	60	do	83.0

Lengthening the outlet tubing caused an increase in the major deposit until a maximum was reached. In the case of clay No. 1 the rate of increase of deposit was less for paper tubing than for metal tubing, although both types were of the same diameter. The maximum percentage of deposit in each case, however, was approximately the same. It is not known whether the increased deposit arises as a result of friction with the walls of the outlet tube, or by a redistribution of charges from particle to particle, or both.

The diameter of the outlet tubing and the presence of a coating of material probably affect the amount of deposit, as was indicated by the fact that whenever the outlet tube became partly clogged with powder, the major deposit was considerably reduced. For example, when the 20-inch iron outlet tube was allowed to become about half filled throughout its length with talc No. 2, the percentage of dust deposited on the positive plate dropped from 80.0 to 56.0 percent.

The size of the feeder opening also affects the ratio of deposits obtained, assuming that the air stream is held constant. The feeder opening on the duster was graduated in eighths and the deposit at each setting was determined. Precautions were taken to turn the crank at a uniform rate. The deposits shown in table 6 were obtained with bentonite clay No.3 and 12 inches of iron outlet tubing was used.

TABLE 6.—*Variation in dust deposit with different feeder openings*

Feeder setting	Proportion of total deposit on positively charged plate	Feeder setting	Proportion of total deposit on positively charged plate
	Percent		*Percent*
One-eighth open	92.7	Five-eighths open	64.3
One-fourth open	80.9	Three-fourths open	64.2
Three-eighths open	78.2	Seven-eighths open	62.3
One-half open	64.1	Entirely open	60.1

In commercial dusting, powders are seldom used singly, dusting materials usually being compounded in mixtures of two, or occasionally three, powders. Consequently, the question arises as to the effect on the ratio of deposits when two powders are blown from the duster simultaneously and there is opportunity for friction between the particles of unlike powders. Four mixtures of two powders each were tested and the results are given in table 7.

TABLE 7.—*Deposits obtained when mixtures of two powders were tested*

Mixture	Charge on plate	Proportion of clay	Proportion of total deposit
		Percent	*Percent*
Bentonite clay No. 2 and redwood flour No. 1	Negative	0	94
	do	1	87
	do	5	70
	do	14	50
	Positive	25	60
	do	31	63
	do	35	70
	do	55	80
	do	57	83
	do	62	84
	do	86	89
	do	95	91
	do	99	95
	do	100	92
		Talc	
Talc No. 1 and walnut-shell flour No. 2	Negative	0	70
	do	1	86
	do	5	76
	Positive	50	54
	do	95	63
	do	99	68
	do	100	71
		Calcium carbonate	
Calcium carbonate No. 4 and diatomite No. 2	do	0	81
	do	1	81
	do	5	72
	do	50	70
	do	95	60
	do	99	55
	do	99.8	55
	Negative	100	53
		Walnut-shell flour	
Bentonite clay No. 1 and walnut-shell flour No. 2	Positive	0	93
	do	1	53
	do	5	68
	Negative	50	58
	do	95	62
	do	99	72
	do	100	70

Two noteworthy points are brought out in this test. In four out of eight cases the incorporation of 1 percent of another powder increased the deposit of the major component. Furthermore, the deposit which will be obtained with a mixture of two powders cannot be predicted from the behavior of each component studied separately (tables 2 and 7).

SUMMARY

To study deposits of commonly used insecticidal dusts and diluents a device was constructed with which the deposits of materials on positively and negatively charged plates could be measured. As a result of these tests it has been shown that:

In general, powders of plant origin gave heavy deposits on the negatively charged plate.

Diatomites and clays gave heavy deposits on the positively charged plate.

All other materials were variously distributed, a feature which was apparently influenced by the composition of the particular dust.

The material of which the outlet tube was constructed influenced the ratio of the deposits on the negative and positive plates.

An increase in the length of outlet tubing increased the major deposit of any given material up to a maximum point beyond which no further increase or decrease was apparent.

As the feeder opening was increased the major deposit of bentonite clay No. 3 decreased.

The addition of 1 percent of one powder to another increased the major deposit in four out of eight cases studied.

The deposit that would result from a mixture of two powders could not be predicted from a study of each of the component materials.

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BORON DEFICIENCY IN GARDEN AND SUGAR BEET [1]

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INTRODUCTION

The heart and dry rot of mangel and sugar beet (*Beta vulgaris* L.) was shown by Brandenburg (*5*)[2] in 1931 to be due to a deficiency in available boron. This work has been confirmed by numerous investigators, and much of the recent literature on the subject has been reviewed by Dennis et al. (*9, 10, 11*) and Brandenburg (*6*). The internal black spot of garden beet has become increasingly serious during the past 10 years in various parts of the United States, particularly in those areas in the Northern States where the crop is grown for canning. This disease has already been described (*21*) and the symptoms, therefore, need not be reviewed here. In previous publications (*1, 21*) the possibility of the relation of boron deficiency to this malady was pointed out. About the same time similar indications were secured in New York (*20*), and more recently confirmatory results were published from Oregon,[3] Michigan (*7*), and New York (*19*). The present paper is a report of investigations which have been under way in Wisconsin since 1937 and of which several preliminary reports have been made (*1, 21, 22, 23, 24*).

METHODS AND MATERIALS

In order to study critically the relation of boron to the development of the garden beet, controlled nutrient experiments were conducted in quartz-sand cultures in the greenhouse. An adaptation of the continuous-drip method of Allison and Shive (*2*) was used. The standard complete nutrient solution was a modification of Shive's Best three-salt solution.[4] It was diluted with 10 parts of distilled water for use in the drip system, and a continuous flow of air was provided in the reservoir containing the solution. Clay pots used to contain the quartz sand were varnished before filling; boronfree glassware and as pure chemicals as were available were employed.[5] The complete solution when diluted contained approximately 0.75 p.p.m. of boron. The boronfree solution was similar in every way except for the omission of the boric acid. Seed was sown directly in the sand, being supplied with either the complete or the boronfree nutrient. When the effect of change from the complete to the deficient solution and vice versa was to be studied, all plants were first removed

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[2] Italic numbers in parentheses refer to Literature Cited, p. 122.

[3] POWERS, W. L., and BOUQUET, A. G. B. USE OF BORON IN CONTROLLING CANKER OF TABLE BEETS. Oreg. Agr. Expt. Sta. Cir. of Inform. 195, 6 pp., illus. 1939. [Processed.]

[4] The formula used was as follows: KH_2PO_4, 0.018 M; $Ca(NO_3)_2.4H_2O$, 0.0052 M; $MgSO_4.7H_2O$, 0.015 M; traces of $CuSO_4.5H_2O$, $FeSO_4 7H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_3.

[5] The writers are indebted to Dr. Kermit C. Berger, Department of Soils, for testing the chemicals and the water supply for the presence of boron.

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from the "complete" and "boronfree" pots. A certain number were then transplanted to the original pots and the remaining ones were exchanged and then transplanted. Similar handling of all plants was thus obtained. When the effect of boron starvation at later stages was to be studied, plants were grown to the desired stage in the complete nutrient solution and the entire pot was then transferred to the boron-deficient nutrient solution.

Field studies were carried out in several locations in Wisconsin. Land for this purpose was usually selected in fields which had shown heart rot of sugar beet or internal black spot of garden beet in previous crops. In determining the extent of internal black spot of garden beet in the field experiments two criteria were used. Random samples of 25 or 50 plants were taken from each treatment plot. Each root was cut in slices approximately one-quarter inch in thickness and each piece examined for the disease. The percentage of plants showing any sign of disease was recorded as the first criterion. The beets were, in addition, placed in one or another of four classes which represented grades of disease: i.e., clean, slight, moderate, severe. By giving these classes specified weights a disease index was calculated which constituted the second criterion. The weights given to the different classes were as follows: Clean, 0; slight, 20; moderate, 50; severe, 100. To secure the disease index, the number of plants in each class was multiplied by the class weight and the sum of these figures was divided by the product of the total number of plants.

Boron was applied as commercial borax,[6] usually incorporated in the fertilizer. This mixture was applied either broadcast or in drills in a separate furrow 1½ to 2 inches at one or both sides of the row and slightly deeper than the seed. Broadcast applications were made with a fertilizer sower and the materials were disked into the soil immediately.

EXPERIMENTAL RESULTS

GREENHOUSE STUDIES

Plants in Sand-Nutrient Cultures

Seedlings appeared 4 to 6 days after seed was sown in sand cultures. When the nutrient solution was free from boron from the beginning, deficiency symptoms appeared promptly on the young unfolding leaves. An intensification of the red pigment was commonly perceptible first along the midrib and veins and then at the tip or on one side of the leaf. Some unilateral development occurred, and reduction in growth rate was apparent. In figure 1, seedlings of two varieties, Detroit Dark Red (Ferry strain) and Good For All, are shown 31 days after sowing in normal and in boron-deficient cultures. Stunting was much more pronounced in the Good For All variety, which is in conformity with its greater susceptibility in the field to be described later. If boron-starved plants were continued longer in boronfree nutrient, growth was very slow and the plants changed relatively little in appearance, although a large percentage of them died in the course of several weeks.

When plants grown for 30 to 40 days in normal and boron-deficient solutions were interchanged, acute symptoms soon appeared in the

[6] Most of the borax for these experiments was supplied by the Pacific Coast Borax Co.

healthy plants while the deficient plants recovered rapidly. This was shown in the same two varieties in figure 2. In the Detroit Dark

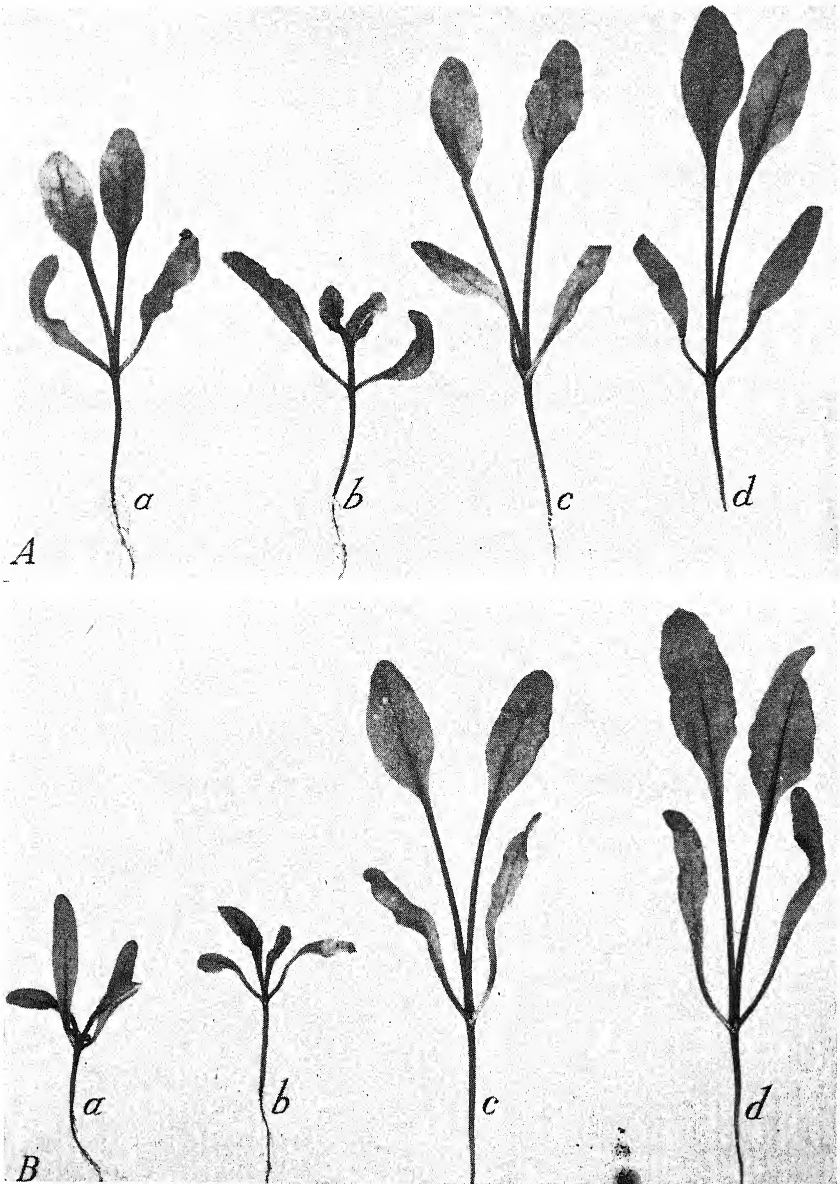

FIGURE 1.—Beets grown from seed in sand-nutrient cultures for 31 days. *A* Detroit Dark Red variety (Ferry strain); *B*, Good For All variety. In both *A* and *B*: *a*, *b*, plants grown in boronfree nutrient; *c*, *d*, plants grown in complete solution.

Red plant started in normal solution and transferred to the deficient solution (fig. 2, *A*, *c*) the youngest leaves remained dwarfed and the growing tip died, although little change but that of deeper red color

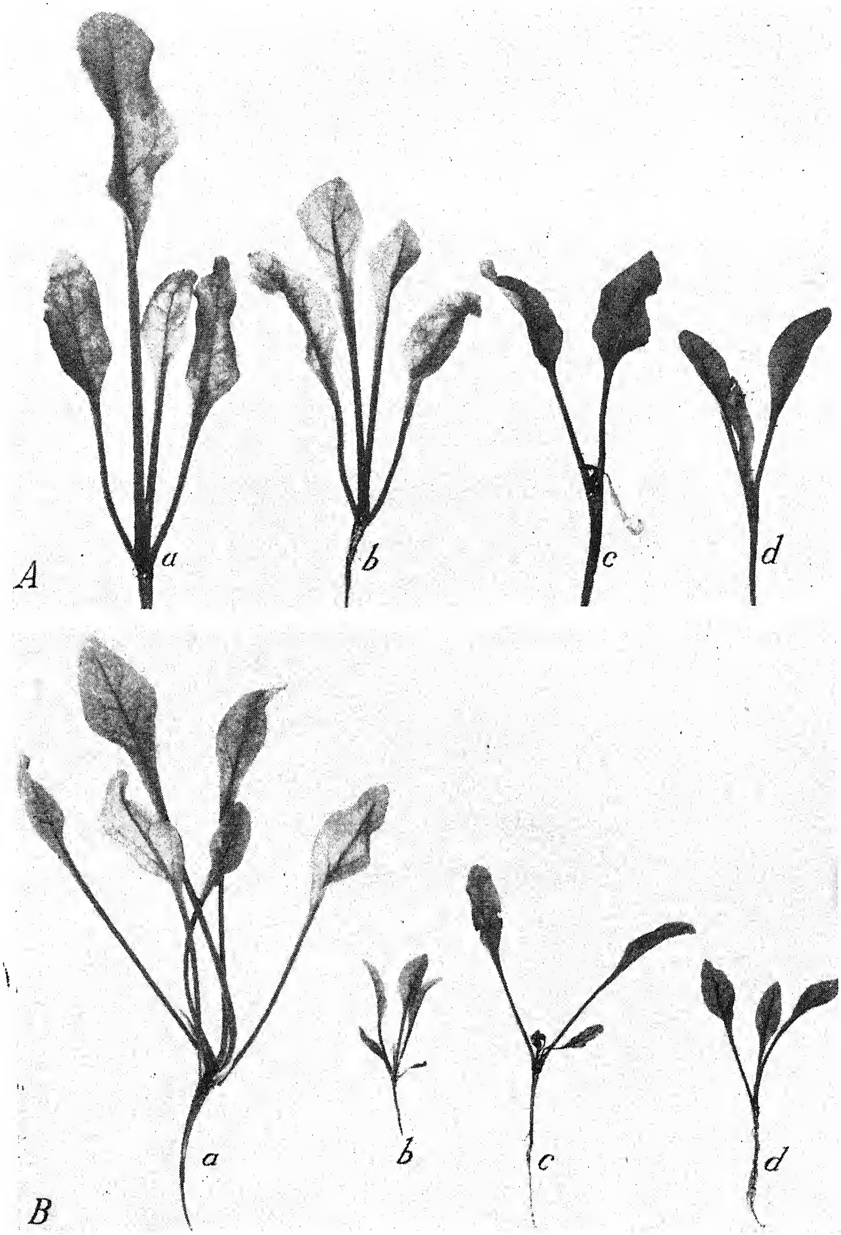

FIGURE 2.—Beets grown in sand cultures in complete solution for 38 days and then transferred to boronfree nutrient for 22 days, and vice versa. *A*, Detroit Dark Red variety (Ferry strain); *B*, Good For All variety. In both *A* and *B*: *a*, plant grown in complete solution for 60 days; *b*, plant grown in boronfree solution for 38 days, then in complete solution for 22 days; *c*, plant grown in complete solution for 38 days, then in boronfree solution for 22 days; *d*, plant grown in boron-deficient solution for 60 days.

occurred in the first two leaves, which were nearly mature when the transfer was made. This behavior is in contrast with that of the plant grown continuously in boron-deficient nutrient (fig. 2, *A*, *d*) which was dwarfed early and, growing more slowly, required less boron than the one growing vigorously in the normal solution when it was suddenly deprived of this element (fig. 2, *A*, *c*). It would appear that the boron acquired by the first two leaves in this plant was rapidly fixed and as already reported by Brandenburg (*6*), was not subject to translocation to the growing point. The plant grown in boron-deficient solution for 38 days (fig. 2, *B*, *d*) had its original meristem intact and consequently a plant similarly treated (fig. *2*, *A*, *b*) commenced normal growth when transferred to the normal solution.

The response of Good For All plants was of the same order as that of Detroit Dark Red. However, the effect of boron-deficiency was greater at 38 days in this more susceptible variety (fig. 2, *B*, *d*). The recovery of the deficient plant was less rapid (fig. 2, *B*, *b*) and the collapse of the plant transferred from normal to deficient solution was more prompt (fig. 2, *B*, *c*).

When plants were allowed to attain greater size in the normal solution before boronfree nutrient was applied or when a trace of boron was left in the nutrient, symptoms developed more slowly and the disease picture was somewhat more suggestive of the usual appearance of the disease in the field. In figure 3 are two plants grown for 17 days from seed in the complete solution, then watered with a reduced-boron nutrient. Boron-deficiency symptoms did not appear until 43 days later, and the photograph was taken after another 50 days had elapsed. In figure 3, *A*, the first three leaves were normal in size and color although chlorosis in the oldest leaves sometimes occurred. The next two leaves were normal in size but the red pigment was intensified somewhat, while the next younger leaves showed malformation typical of boron deficiency. In figure 3, *B*, the first four leaves were normal, the next two were somewhat deeper in color, while the seventh leaf showed typical unilateral malformation. The next younger leaves appeared quite normal, possibly as a result of the release of boron from dying leaves and roots which had become incorporated in the sand.

When beet plants were grown in the complete solution long enough to permit taproot enlargement, boron starvation brought on necrosis, usually near the periphery, in the rings in which bundles had been differentiated most recently. Sometimes, however, extensive darkening was produced throughout the center of the beet. In figure 4 are the cross sections of roots of two plants grown in nutrient-sand cultures for 145 days. The larger root, free from necrosis, was grown in complete solution throughout the period. The smaller root was grown on complete solution for 71 days, then in boronfree nutrient for 74 days. The black corky tissue is not unlike that found in beet roots affected with internal black spot in nature.

Beet roots showing typical internal black spot were collected in the field and halved longitudinally. Corresponding halves were placed in normal and in boron-deficient nutrient in sand cultures. Extensive development of normal leaves ensued in the former, although no change in the necrotic areas of the fleshy root occurred. In the latter, stunted malformed leaves formed and died, only to be followed by others arising from dormant buds at the bases of the

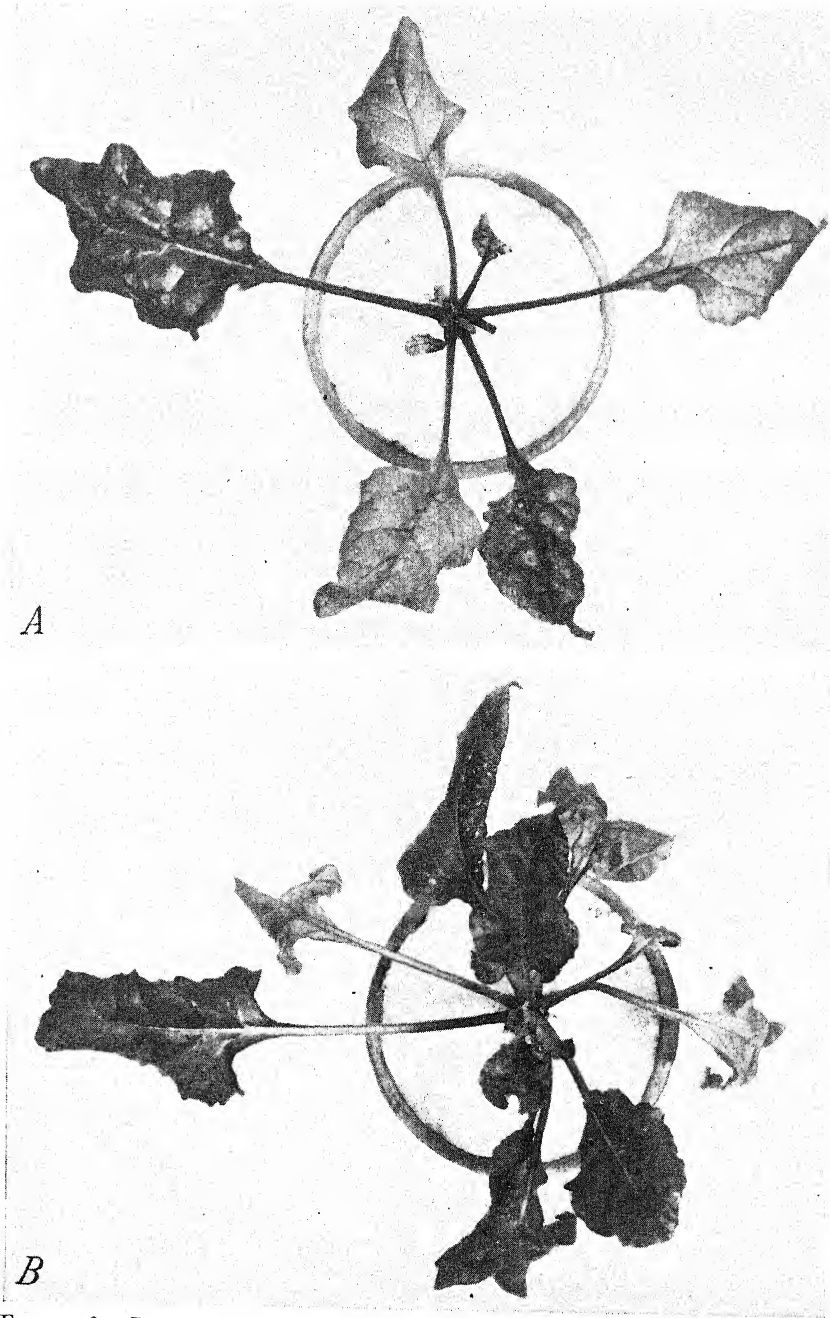

FIGURE 3.—Beet plants of Detroit Dark Red variety (Ferry strain) grown from seed for 17 days in sand culture with complete nutrient solution, then with a solution containing traces of boron for 93 days. See further explanation in the text.

original ones, leading eventually to a rosette of malformed and dead leaves. Such a pair of plants is shown in figure 5.

When beet roots grown in the field with an adequate supply of boron were planted after a period of dormancy, in boronfree nutrient sand cultures, leaf and floral stem development was at first normal, but eventually the apical leaves showed symptoms of boron

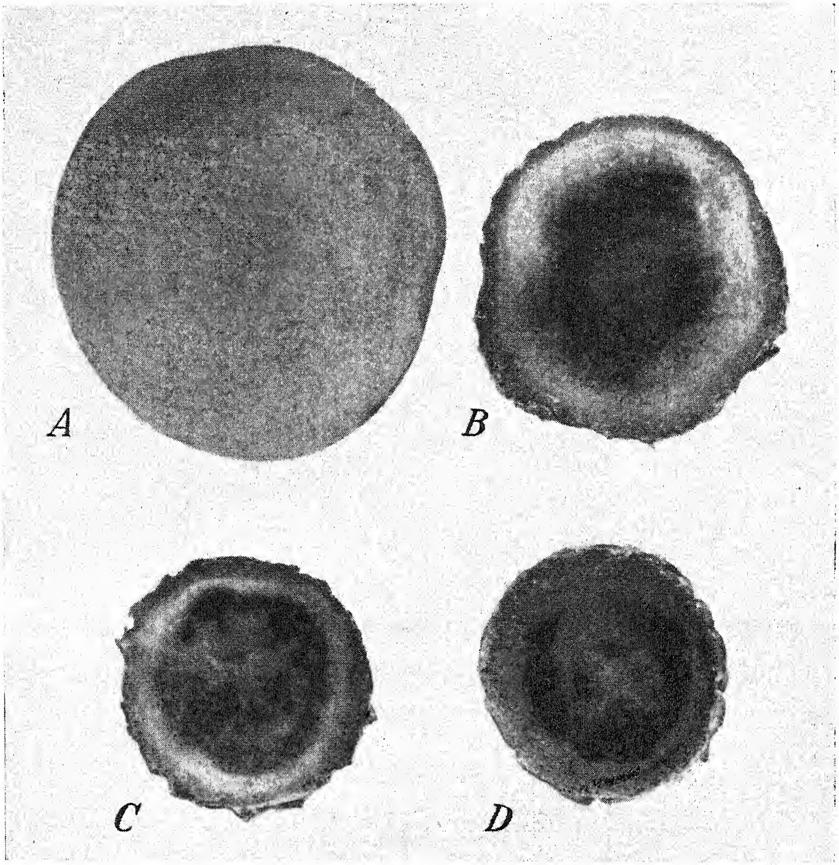

FIGURE 4.—*A*, Cross section of the root of a beet plant grown from seed in complete-nutrient sand culture for 145 days. *B*, *C*, *D*, Cross sections of the roots of beet plants grown concurrently with that in *A* in complete solution for 71 days, then in boronfree nutrient for 74 days.

deficiency. Frequently elongate fissures developed in the stem, sometimes extending its full length. Gradual reduction in growth generally followed. These phases of the disease are illustrated in figure 6.

PLANTS IN SOIL

The appearance of symptoms of boron deficiency in garden beets grown in sand-nutrient cultures was compared with that of plants grown in the greenhouse in the winter months at Madison, Wis., on soil collected from fields in which severe internal black spot had occurred. It was at first postulated that a simple greenhouse pot

test might be devised in which the relative availability of boron in the soil concerned might be estimated as a guide to field requirements

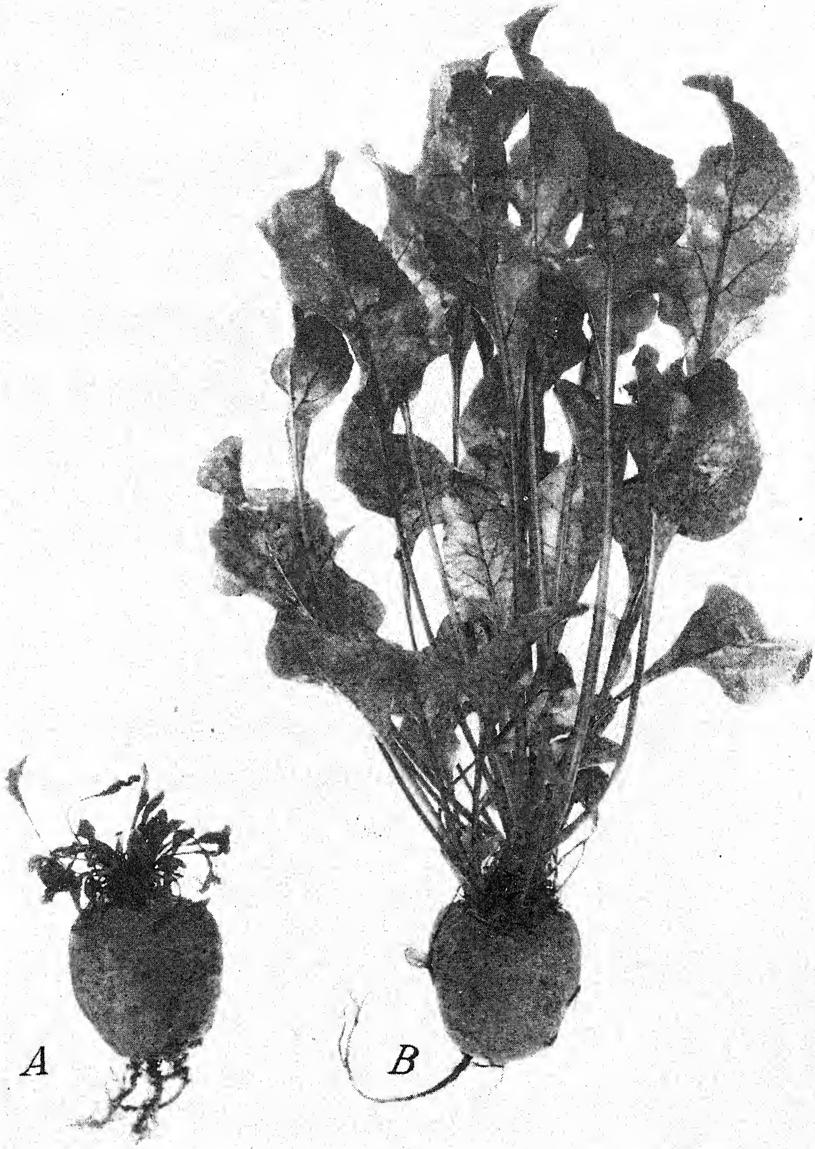

FIGURE 5.—A beet root showing internal black spot when collected in the field at maturity was split longitudinally and one part (*A*) was placed in sand culture with a boronfree nutrient, while the other (*B*) was placed in sand culture with a complete solution. Photographed 87 days later. See further explanation in the text.

for correction of the deficiency. In general the acute boron-deficiency symptoms described above in the sand-nutrient cultures were absent,

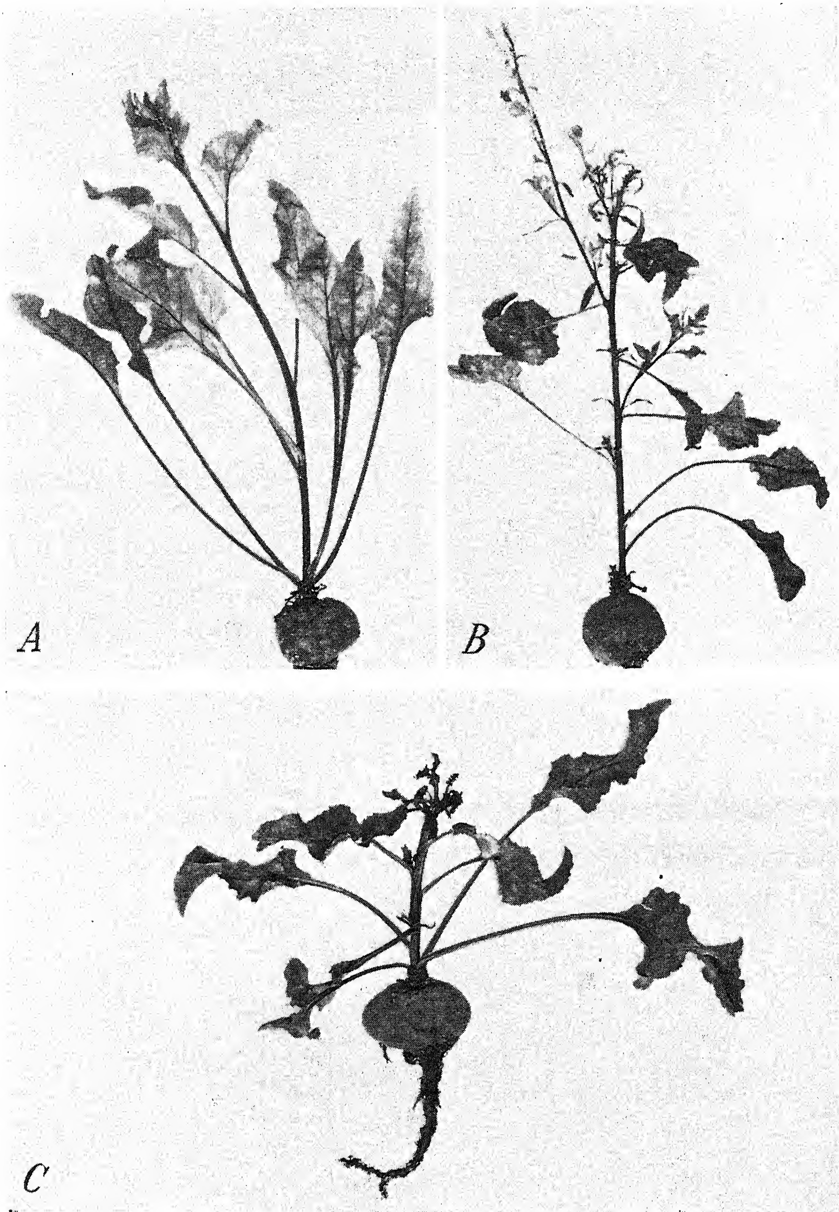

FIGURE 6.—Beets grown in the field with an adequate boron supply, stored at 5° C. for 60 days, planted in sand-nutrient cultures and photographed 60 days later. *A*, plant grown in complete nutrient; *B*, *C*, plants grown in boronfree nutrient. Note advanced flower development in *B* and *C*, stunting of terminal bud in *B* with consequent development of floral stems from leaf axes, and stunting and elongate stem cankers in *C*.

except for somewhat greater intensification of red pigment and reduced vigor. Unilateral leaf development, cross hatching of the petiole, apical bud necrosis, and rosette of the tops appeared rarely or only after a period of several months. When various amounts of borax were added to such soils the growth of the plants was usually enhanced and normal color of the foliage returned. The results of many such comparisons indicated that soils in which garden beets were severely affected were not completely free from available boron but sufficiently so at the time of greatest need by the growing crop to bring on deficiency symptoms. It was also indicated that under greenhouse management of soil where moisture was more uniform, there was not the extreme development of boron-deficiency symptoms as sometimes observed in the same soil under certain field conditions. The plant symptoms which resulted were therefore those of moderate but protracted deficiency.

The symptoms of boron deficiency in garden beets in the field already described (*21*) may be regarded as the combination of effects produced in the greenhouse in soil and in sand-nutrient cultures. The crop planted on boron-deficient soil in Wisconsin in May or early June grows quite normally insofar as general appearance is concerned until late July or early August. Some intensification of red pigment or yellowing of older leaves may occur, but this may result from various other environmental influences and it does not serve as a consistent means of diagnosis. In August and September the disease appears in tops and roots, the extent and severity again being greatly influenced by climatic environment. If the soil is acutely deficient in boron the greater need of the enlarging plants results in the regular appearance of symptoms. If the boron supply is marginal, distinguishable top symptoms may fail to appear or they may consist only of slight interveinal yellowing. On the other hand, in marginal cases sudden appearance of extensive and acute leaf and root symptoms may occur. This type of disease development is commonly associated with periods of drought. However, the writers' observations have shown that heavy rainfall starting sudden new growth activity after a protracted dry spell is probably the most effective incitant of severe internal black spot. It is possible that during protracted dry spells the already low supply of available boron in the soil solution is rendered temporarily less available and that concomitantly the fibrous root system of the plant is rendered less effective. When under such circumstances suddenly stimulated growth of plants approaching maximum development is incited by an abundant water supply, the symptoms which follow are most nearly like those produced in sand-nutrient cultures by extreme deficiency of boron.

FIELD STUDIES

Certain preliminary field experiments in 1937 have already been reported (*21*). These were side-row applications made when the plants were in the third- or fourth-leaf stage. They indicated a tendency for black spot to be reduced, although in none of the fields used in this series was the disease very severe. In the late summer of 1937 a limited survey was made of fields of garden beet in Wisconsin in which black spot was present. Other surveys were made in 1938, 1939, and 1940. The results are summarized in table 1. More than

60 percent of the fields examined critically during this 4-year period were neutral or alkaline in soil reaction, but more than 15 percent were within the range of pH 5.2 to 5.5. About the same number of fields in which the disease was rated as severe were in low areas as in high upland tracts. It had been already noted in 1937 (*21*) that no

TABLE 1.—*The occurrence of internal black spot of garden beets in certain commercial fields in Wisconsin, 1937–40*

Field No.	Kind of soil	Topography of portion of field examined	pH of soil [1]	Severity of disease	Year
1	Light sandy loam	Hillside	8.0	Medium	1938
2	Light silt loam	Level upland	8.0	do	1938
3	Dark sandy loam	do	8.0	Severe	1938
4	Dark loam	Lowland	8.0	Medium	1938
5	do	do	8.0	Severe	1938
6	Light loam	Hillside	8.0	do	1938
7	do	do	8.0	do	1938
8	Dark silt loam	Lowland	8.0	do	1939
9	Light sandy loam	Level upland	8.0	do	1939
10	Dark silty clay loam	Low	7.9	do	1937
11			7.9	do	1937
12		High and low	7.8	do	1937
13	Dark silt loam	Low	7.8	do	1937
14	do	do	7.7	do	1937
15	Medium fine sandy loam	High	7.5	Slight	1938
16	Dark silt loam	Low	7.5	Severe	1939
17	do	Level upland	7.5	do	1940
18	Medium silt loam	Upland	7.5	do	1940
19	Dark silt loam	Swale	7.2	do	1939
20	Medium silt loam	Hillside	7.2	do	1939
21	Light sandy loam	Level upland	7.2	do	1939
22	Light fine sandy loam	Hillside	7.0	Medium	1938
23	do	Hilltop	7.0	do	1938
24	Dark silt loam	Low	7.0	Severe	1939
25	do	do	7.0	do	1940
26	do	Hillside	6.8	do	1939
27	do	Low	6.7	do	1939
28	Light sandy loam	Level upland	6.5	do	1938
29	Peat	Low	6.3	do	1939
30	Medium silt loam	Hillside	6.2	Medium	1937
31	Medium sandy loam	Level upland	6.2	Severe	1939
32	Light silty loam	do	6.1	do	1938
33	Medium silt loam	Low	6.0	Medium	1937
34		High	5.5	Severe	1937
35		do	5.5	Medium	1937
36	Light sandy loam	Level upland	5.5	do	1940
37	do	do	5.5	Severe	1940
38	do	Hillside	5.4	do	1939
39	do	Hillside and hilltop	5.4	do	1940
40	do	Level upland	5.2	Medium	1940

[1] The determinations of soil reaction and kind of soil were kindly made by Dr. Harold H. Hull, Department of Soils, University of Wisconsin.

correlation existed between the occurrence of internal black spot of garden beet and the topography of the soil, and this was borne out when the survey was extended 3 years longer. While a preponderance of the disease occurs on neutral or alkaline soil, it is not necessarily restricted to such soils in Wisconsin.

FIELD EXPERIMENTS AT WINNECONNE, WIS.

The field used at Winneconne, Wis., consisted of poorly drained Poygan silty clay loam, relatively high in organic matter and for the most part slightly alkaline in reaction. Broadcast applications were made in the spring of 1938 on a series of plots in this field, and the disease reaction on beets was studied in three successive seasons, 1938, 1939, 1940. Side-of-row applications were made in another series of row plots. Since manganese deficiency was suspected in this soil, application of manganese sulphate was made in some plots alone and

with borax. The salts of various other minor elements were also included to determine any possible influence they might have on the development of the disease. The results of broadcast applications will be discussed first.

Broadcast applications.—Plots 30 by 30 feet in dimension were laid out in the spring of 1938 in four replicate series of nine treatments each, the latter being arranged in random order and each plot surrounded by a 5-foot untreated border.[7] Treatments were made about 2 weeks before sowing. In the spring of 1939 each plot was divided into two equal parts on one of which the treatment of 1938 was repeated. In the spring of 1940 no minor-element treatments were made but a complete fertilizer was applied broadcast just before sowing. In 1938 and 1939 sugar beets, garden beets and Wisconsin Refugee beans (*Phaseolus vulgaris* L.) were planted in each plot. The last species was used because it is regarded as rather sensitive to boron toxicity and it thus served as an index of any detrimental effects of treatment. Cabbage (*Brassica oleracea capitata* L.), cauliflower (*B. oleracea botrytis* L.), and certain other crucifers were also included; the studies with these crops are reported elsewhere (*25*). In the case of garden beet the two varieties Good For All and Detroit Dark Red were used. The same seed stock of the former was planted in 1938 and 1939. Although seed of the second variety was secured each season from the Ferry-Morse Seed Co. of Detroit, Mich., it was later realized that the so-called Ferry strain of Detroit Dark Red was used in 1938 and the Morse strain of that variety in 1939. It was also learned later that the Morse strain is more susceptible to boron deficiency than the Ferry strain, and consequently the results of the two seasons are to be compared with that fact in mind.[8] In 1940 only sugar beets were planted on these plots. Seed was sown on May 25 in 1938, on June 20 in 1939, and on July 5 in 1940.

Stand counts of sugar beet and garden beet were taken on all plots in 1938 and 1939. No significant decrease in stand in any of the treated plots was noted. While there was a slight increase in stand in many of the treated plots in each year the average increase was not statistically significant for any treatment. No toxic effect of any of the treatments was evident in either year except for slight marginal chlorosis on the lower leaves of an occasional bean plant in the 60-pound-per-acre application of borax. This was decidedly a transient effect and there was no evidence of permanent suppression or reduction in yield.

The occurrence of heart rot in sugar beet on these plots in 1938, 1939, and 1940 is recorded in table 2. On the untreated plots the plants with heart rot of the tops averaged 18 to 56 percent. The 100-pound treatment with manganese sulfate did not alter the incidence of heart rot although it stimulated top growth perceptibly in some plots. The application of 20 pounds of borax per acre with or without the incorporation of manganese or other minor elements was sufficient to eliminate heart rot completely in 1938. Moreover, the carry-over effect of the borax treatments in 1939 was sufficient to prevent the disease in that year. In 1940 the 40- and 60-pound treatments made 3

[7] The writers are indebted to Dr. J. H. Torrie, Department of Agronomy, University of Wisconsin, for advice in the arrangement of these and other plots discussed in this paper and in the statistical analysis and interpretation of results.

[8] The Morse strain of Detroit Dark Red is considered by Magruder et al. (*15*) to be so different from the original type that they refer to it as a distinct variety, under the name Morse Detroit.

years previously were still effective while those made 2 years before were all effective. However, in the 20-pound-per-acre treatment of 1938 the available boron was so low in 1940 that some disease developed. It may be concluded that in this soil, boron was retained in available form for three crop seasons, but that in the third season it was again becoming insufficient for normal development of sugar beets at the 20-pound level, through depletion, leaching, or fixation in an unavailable form.

TABLE 2.—*The effect of broadcast applications of borax and of salts of certain other minor elements on the incidence of heart rot in sugar beets grown in boron-deficient Poygan silty clay loam near Winneconne, Wis., in 1938, 1939, and 1940*

Treatment		Plants showing heart rot (average of 4 replicates) in—				
		1938	1939		1940	
Borax per acre	Manganese sulfate per acre	Treated in 1938 only	Treated in 1938 only	Treatment repeated in 1939	Treated in 1938 only	Treatment repeated in 1939
Pounds	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
[1] 0	0	18	25	34	50	56
0	0	28	26	35	23	30
0	100	28	47	31	34	35
20	0	0	0	0	2	0
20	50	0	0	0	0	0
20	[2] 50	0	0	0	9	0
40	0	0	0	0	0	0
40	50	0	0	0	0.5	0
60	0	0	0	0	0	0

[1] No fertilizer was applied in this plot; in all other plots 3-12-12 fertilizer was added at the rate of 450 pounds per acre in 1938, and 300 pounds in 1939 and 1940.
[2] There was also added per acre in this plot: 50 pounds $CuSO_4.5H_2O$, 50 pounds $ZnSO_4.7H_2O$, 50 pounds $FeSO_4.7H_2O$, and 100 pounds NaCl.

The parallel results in 1938 and 1939 with red beets are given in table 3. In order to study the progress of diseases, three sets of random samples were taken from each plot in 1938 at approximate 2-week intervals, beginning on July 29. There was with both varieties a decided increase both in percentage of disease and in disease index from July 29 to August 15, but there was little change from August 15 to August 31. Throughout the data the severity of the disease as indicated by the disease index was correlated closely with the percentage of diseased plants. The disease was reduced significantly in all treatments containing borax as compared with the untreated plots. It is to be noted that although heart rot of sugar beet was completely eliminated by the borax treatments, internal black spot of garden beet was present to some degree in most of the treatments. It is thus evident that the latter type is more susceptible to boron deficiency than the former and that Good For All, as already shown in the sand-nutrient studies, is more susceptible than Detroit Dark Red (Ferry strain). Although there was a trend toward less disease with increase in the rate of borax applied, the differences between borax treatments were not usually significant. The 100-pound treatment with manganese sulfate, as with sugar beets, had no effect upon internal black spot, although it did perceptibly stimulate growth. In the 20-pound borax treatment the disease was increased when 50 pounds per acre of manganese sulfate was added. This may have been due to the fact that growth was stimulated somewhat

TABLE 3.—*The effect of broadcast applications of borax and of salts of certain other minor elements on the incidence (percentage) and severity (disease index) of internal black spot in garden beets grown in boron-deficient Poygan silty clay loam near Winneconne, Wis., in 1938 and 1939* [1]

Treatment		Good For All variety in—										Detroit Dark Red variety [2] in—									
		1938: Notes on—						1939: Notes on Aug. 13				1938: Notes on—						1939: Notes on Aug. 13			
		July 29		Aug. 15		Aug. 31		No further Treatment		Treatment repeated		July 29		Aug. 15		Aug. 31		No further treatment		Treatment repeated	
Borax (Pounds per acre)	Manganese sulfate (Pounds per acre)	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index	Plants diseased	Disease index
		Per-cent		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*		*Per-cent*	
0 [3]	0	17	4.7	51	21.3	70	35.0	73	58.4	68	51.4	8	2.6	12	5.5	19	7.9	77	65.0	73	56.2
0	0	29	12.7	54	32.0	56	23.9	59	48.7	68	52.5	15	4.6	55	25.4	44	13.0	68	47.0	86	79.8
0	100	22	6.6	62	35.1	63	27.7	50	42.1	68	43.2	10	3.9	40	20.5	44	17.4	56	50.5	33	25.5
20	0	2	.3	16	3.2	20	5.8	15	8.4	5	2.8	1	.2	9	2.4	6	1.9	16	11.4	4	2.2
20	50	2	.3	22	5.9	22	8.4	10	6.1	14	6.1	4	1.9	14	4.2	8	3.4	18	12.6	11	6.7
20	50 [4]	7	1.8	19	6.9	22	8.8	40	20.8	9	4.7	3	1.0	8	1.6	10	2.9	19	9.0	11	4.8
40	0	0	.0	12	2.3	18	5.7	9	4.6	2	1.3	0	.0	1	.2	5	1.2	7	1.8	0	.0
40	50	2	.8	6	1.1	21	3.6	4	1.5	7	2.5	0	.0	3	.5	7	2.1	12	5.8	1	.2
60	0	0	.0	6	1.3	15	3.7	4	1.5	2	.6	0	.0	9	4.0	4	.8	3	1.8	2	.9
Difference required for significance (19:1):																					
Between nonborax treatments		28	12.2	29	17.9	27	11.4	32	37.9	17.0	17.8	15	5.5	29	15.3	27	12.9	51	58.0	47	46.4
Between borax treatments		(5)	(5)	16	6.2	19	5.8	18	11.9	7.0	3.5	(5)	(5)	12	4.6	7	3.0	14	11.9	10	6.6

[1] The percentages and indices in the table are averages from random samples in each of 4 replicates.
[2] Ferry strain used in 1938; Morse strain used in 1939.
[3] No fertilizer was applied in this plot; in all other plots 3-12-12 fertilizer was applied at the rate of 450 pounds per acre in 1938 and 300 pounds per acre in 1939.
[4] There were also added per acre: 50 pounds $CuSO_4.5H_2O$, 50 pounds $ZnSO_4.7H_2O$, 50 pounds $FeSO_4.7H_2O$, and 100 pounds NaCl.
[5] Since the presence of a number of treated plots with no diseased beets would introduce a bias in the statistical analysis of the data, the minimum significant difference was not calculated.

by the manganese, and presumably the need for boron was correspondingly increased. At the 40-pound-per-acre borax treatment the manganese treatment did not affect the disease reading, possibly because there was a more nearly adequate supply of boron available. At the 20-pound level the addition of salts of several other minor elements with the manganese sulfate did not affect the disease readings.

It may be concluded from this experiment that the internal black spot of garden beet was greatly reduced by application of borax and that the effect was carried over to the second year in this soil, inasmuch as the readings in 1939 for Good For All were essentially the same in plots receiving no further treatment as in those treated again in that year. In Detroit Dark Red most plots showed higher disease readings in 1939 than in 1938. This is undoubtedly due in part to the variation in susceptibility of the two strains of that variety. It emphasizes the importance of care in the selection of the seed stock used in conducting studies of boron deficiency.

It is also important to compare the results of garden beet with those of sugar beet. In the latter, heart rot was completely controlled by 20 pounds of borax per acre, while in the former 60 pounds did not completely correct black spot. It should, of course, be recognized that the comparison is concerned with the symptoms in the leaves of one plant and the symptoms in the roots of the other. A fairer basis might have been had if the roots of sugar beet had been cut and examined also.

Detailed notes of top symptoms on garden beet were taken. They are not presented in detail here, but it is worthy of mention that while top symptoms seemed as a rule to appear slightly in advance of root symptoms, they were, nevertheless, not necessarily concomitant with them. An appreciable percentage of roots showed black spot without recognizable top symptoms, and vice versa. It is possible that symptoms in the tops sometimes appear during a temporary shortage of boron and that normal growth is resumed before macroscopic root symptoms occur.

During the course of these experiments soil samples from representative areas were taken and analyses for available boron conducted by Berger and Truog (*3*). Their results showed a rather close correlation between available boron and the occurrence of black spot n this particular series of plots.

Side-of-row application.—In 1938 a series of plots was laid out in which borax and the salts of other minor elements were applied as side bands. These materials were mixed with the fertilizer and placed in bands at both sides of and 1½ inches away from the row and at the same depth as the seed. The results are given in table 4. None of the treatments was injurious to the stand. There was no significant difference between the percentages of diseased plants in the plots treated with any of the materials, except borax, and the untreated plots. Zinc sulfate appeared to reduce the disease index significantly but the percentage of diseased plants was nearly as high as that of the control. Since this material was used in combination with the salts of other minor elements in many other experiments without any effect upon the disease, this result is not considered significant. The 15-pound treatment with borax was not very beneficial, but beginning with 25 pounds per acre, the disease was reduced consistently

as the amount of this material increased. It was thus evident, as in the broadcast treatments, that boron was the only one of the elements used which had a beneficial effect in controlling internal black spot. It was also shown that under the conditions of these experiments the results were of the same order when the material was applied in concentrated bands at the sides of the row as when it was applied broadcast. The stand was not injured when as much as 80 pounds of borax per acre was applied in this manner. While this heaviest treatment practically eliminated the trouble, there was still an occasional diseased root found, as was the case when 60 pounds per acre was applied broadcast.

TABLE 4.—*The effect of salts of various minor elements on internal black spot of Detroit Dark Red garden beet (Ferry strain) when applied in bands at both sides of the row at time of sowing, Winneconne, Wis., 1938* [1]

Material applied	Amount applied [2] (per acre)	Stand of plants (per yard)	Disease development	
			Plants diseased	Disease index
	Pounds	*Number*	*Percent*	
None		13.4	36.0	22.4
Copper sulfate	50	11.1	46.3	27.0
Ferric sulfate	50	15.6	32.6	11.4
Zinc sulfate	25	13.6	30.6	7.6
Cobalt chloride	20	14.1	45.0	22.8
Manganese sulfate	50	13.9	33.6	10.2
(3)	(3)	16.4	58.5	25.1
Borax	15	14.5	[4] 32.6	6.8
Do	25	13.5	13.6	6.8
Do	35	15.5	11.7	3.6
Do	45	13.9	10.2	3.5
Do	55	13.9	1.6	1.2
Do	65	14.5	2.4	.8
Do	80	12.6	.5	.3
Difference required for significance (19:1):				
Between borax treatments			[4] 8.1	4.0
Between nonborax treatments			30.7	13.3
Between all treatments		5.3		

[1] Seed was sown on May 25; harvest data taken on Aug. 25.
[2] Calculated on the basis of 15 inches between rows.
[3] 50 lbs. $CuSO_4.5H_2O$, 50 pounds; $MnSO_4.4H_2O$, 50 pounds; $FeSO_4.7H_2O$, 25 pounds; $ZnSO_4.7H_2O$, 20 pounds, $CoCl_2.6H_2O$, and 100 pounds NaCl per acre were applied.
[4] The results from the 15-pound-per-acre treatment were not included in the analysis because the variance within this treatment was significantly greater than that within the other treatments.

FIELD EXPERIMENTS AT CLYMAN, WIS.

The treatments conducted at Clyman in 1938 were on Clyde silt loam with a reaction of pH 7.0 to 7.5. They were laid out on a somewhat more extensive scale than those at Winneconne and were designed to provide data on yield as well as on disease development. The beets grown on the area used in 1938 had only a negligible amount of disease, and the results were therefore confined to yield. A split-block experimental design was used in which treatments were randomized within varieties. Three replicates were employed. Plots consisting of four rows each were planted with a standard four-row beet drill equipped with an attachment by which the fertilizer and borax or other materials were applied in a band in a separate furrow 1½ inches away from and of the same depth as that in which the seed was placed. Two varieties were used, Detroit Dark Red (Ferry strain) and Good For All. Fertilizer (3–12–12) was applied in all plots at the rate of 150 pounds per acre.

The results of 1938 are given in table 5. It is to be noted that the stand was not injured in any instance, and in the case of Good For All

TABLE 5.—*The effect of borax and salts of certain other minor elements applied along the row at sowing*[1] *on the stand and yield of garden beets grown on Clyde silt loam at Clyman, Wis., 1938*[2]

Application of borax (pounds per acre)	Detroit Dark Red variety[3]		Good For All variety	
	Stand per yard	Yield per acre	Stand per yard	Yield per acre
	Plants	*Tons*	*Plants*	*Tons*
None	14.3	16.3	16.8	13.2
10	16.5	17.5	17.8	13.7
20	16.3	19.0	19.0	13.6
30	18.6	18.5	24.2	15.6
40	14.9	20.0	21.7	15.9
60	14.7	21.0	21.0	14.8
(4)	15.0	18.8	21.9	14.1
Difference required for significance (19:1)	3.4	1.5	3.7	1.5

1 Borax applied in a furrow 1 to 2 inches deep and 1½ inches away from the row.
2 Seed sown May 20; harvest data, taken Sept. 22, was from 3 replicates.
3 Ferry strain secured from Ferry-Morse Seed Co., Detroit, Mch.
4 A mixture of 50 pounds $CuSO_4.5H_2O$, 50 pounds $MnSO_4.4H_2O$, 50 pounds $FeSO_4.7H_2O$, 25 pounds $ZnSO_4.7H_2O$ and 10 pounds $CoCl_2.6H_2O$ per acre was applied.

it was increased significantly, in the three heaviest treatments with borax and in the treatment with minor-element salts other than borax. It is also to be noted that in the case of Detroit Dark Red the yield was increased significantly in all treatments except that of 10 pounds of borax per acre, and in the case of Good For All in all except those of 10 and 20 pounds of borax per acre. This showed rather definitely that even where the boron naturally available in the soil was sufficient to prevent black spot under the growing conditions of that season, the growth of the plant was stimulated sufficiently to produce significant increases in yield. This type of stimulation was not confined to borax since one or more of the materials applied in the mixture of salts of other minor elements also brought significant increases in yield. Since severe black spot occurred in this field in 1937, it is possible that the boron supply was marginal but that growing conditions in 1938 were such that enough boron was available to prevent macroscopic necrosis, though still not sufficient for optimum growth.

The experiments at Clyman in 1939 were laid out with the same plot design in another field of the same type of soil having a reaction of about pH 7.5. Because of the interest in the comparative value of applying borax with the fertilizer in bands or broadcast, the two methods were compared at 20-, 40-, and 100-pound-per-acre levels. The results are given in table 6. A significant reduction in black spot is noted when each treatment is compared with the control.

The disease was greatly reduced in all cases by borax. As in the trials at Winneconne, the percentage of diseased plants and the severity of disease tended to be reduced with increasing amounts of borax, but even at 100 pounds per acre there were still appreciable amounts present in each variety and the differences between treatments were not always significant. The stand was not materially affected by either type of treatment nor by any level of borax. The two methods of treatment had, as a rule, closely similar effects.

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The yields were taken with only one variety, Detroit Dark Red. Although the crop was not so heavy as that at Clyman in 1938, the yields were greater in all treated plots than in the untreated ones. In each of five treatments the difference was greater than that required for significance, while in the other two treatments the difference was nearly great enough to be significant.

TABLE 6.—*The effect of application of borax, broadcast and at the side of the row, on the stand, yield, and internal black spot of garden beet grown on Clyde silt loam near Clyman, Wis., 1939* [1]

Application of borax		Detroit dark red variety [2]				Good for all variety		
Amount (pounds per acre)	Method	Stand per yard	Disease: Plants affected	Disease: Severity	Yield per acre	Stand per yard	Disease: Plants affected	Disease: Severity
		Plants	*Percent*	*Disease index*	*Tons*	*Plants*	*Percent*	*Disease index*
None		14.3	39.6	27.3	6.1	8.2	64.9	51.6
20	Side of row	15.0	10.7	8.1	7.7	7.8	19.1	10.8
20	Broadcast	12.9	18.7	11.1	7.1	7.4	18.5	9.4
40	Side of row	14.8	16.0	11.3	6.9	8.8	14.5	8.4
40	Broadcast	14.7	14.7	10.0	7.2	6.9	14.5	8.6
60	Side of row	14.5	10.7	6.9	8.2	9.0	10.5	3.8
100	do	16.3	10.7	6.7	8.1	10.6	4.6	2.3
100	Broadcast	16.4	5.3	2.9	7.8	7.5	7.2	3.4
Difference required for significance (19:1)		3.8	9.6	7.0	1.1	2.2	7.0	6.1

[1] Seed sown May 25; harvest Oct. 5.
[2] Ferry strain.

FIELD EXPERIMENTS AT ROCKFIELD, WIS.

The experiments at Winneconne and at Clyman were on relatively heavy clay loam or silt loam soils which were rather high in organic matter. No evidence of plant injury or yield depression was noted even with applications as high as 100 pounds of borax per acre. In 1939 a field of Miami silt loam was chosen near Rockfield, Wis., where black spot had been observed on previous crops and in which the soil was somewhat lighter in texture. This was laid out and planted on the same plan as the experiment at Clyman in 1939, in which side-of-row and broadcast applications of various amounts of borax were compared as to their effect on stand, disease incidence, and yield. As shown in table 7, there was neither reduction nor increase of stand as a result of the applications, and no significant effect upon yield. The disease developed relatively late in the season, but by the time of harvest a considerable amount was present in the untreated plots. In this experiment the disease was less in the 20-pound treatment than in the untreated plots, but the decrease was barely significant in the side-of-row application. In this case at the threshold treatment it would appear that the broadcast method was definitely better than the side-of-row method. However, a larger body of data would be needed to demonstrate the point in view of the fact that the difference was not consistent at higher applications in this series nor in the Clyman experiment of 1939. There was less disease at 30, 40, and 60 pounds per acre than at 20 pounds but the differences between the three higher treatments were not consistent with the amount of borax applied nor were they statistically significant. The lowest disease

incidence and index were at 100 pounds per acre applied broadcast but again this heavy treatment did not completely control the disease.

TABLE 7.—*The effect of application of borax, broadcast and at the side of the row, on stand, yield, and internal black spot of garden beet grown on Miami silt loam near Rockfield, Wis., 1939; Detroit Dark Red variety, Ferry strain*

Application of borax		Stand per yard	Disease		Yield per acre
Amount (pounds per acre)	Method		Plants affected	Severity	
		Plants	*Percent*	*Disease index*	*Tons*
None		[1] 19	38.8	28.0	11.3
20	Side of row	20	27.7	18.2	11.1
20	Broadcast	17	[1] 17.0	[1] 7.9	8.9
30	Side of row	21	11.0	5.3	11.4
40	do	17	10.7	4.0	8.8
40	Broadcast	16	14.2	5.8	11.1
60	Side of row	20	15.9	8.1	10.9
100	do	16	9.9	4.4	10.0
100	Broadcast	21	1.4	1.1	10.5
Difference required for significance (19:1)		(2)	18.7	11.3	(2)

[1] Each value is the average of 2 replicates.
[2] Differences not significant.

APPLICATION OF BORAX DURING THE GROWING SEASON

It was observed repeatedly that the symptoms of boron deficiency in both garden and sugar beets usually appeared about midseason. In the data from Winneconne in 1938 (table 4) it was shown that the disease increased rapidly during the first half of August. The question arose as to whether if borax were applied at or slightly before the first appearance of symptoms most of the damage to the crop would be prevented, and whether application at such a time, when the plant was in immediate need of boron, would be more effective than treatment at the time of planting. To obtain information on these points experiments were conducted at Winneconne in 1938, 1939, and 1941. In 1938 seed of Detroit Dark Red (Ferry strain) garden beet was sown on May 25, borax was applied in a trench at the side of the row on July 16, and harvest was completed on September 28. In 1939 Detroit Dark Red (Morse strain) garden beets were sown on June 20, borax in a water suspension was applied over the row on August 3, and harvest was completed on September 14. In 1941 sugar beets were sown on June 7, borax was applied in dry form broadcast on July 14, and the crop was harvested on October 6. In 1938 and 1939 top symptoms of boron deficiency were not apparent at the time of treatment, but they appeared in the controls about a week after treatment. In 1941 a trace of heart rot was present at the time of treatment. The results of these treatments are given in table 8.

Midseason treatments were very effective in all cases. In 1938 the results were about the same as those in the nearby spring applications reported in table 4. In 1939 very effective control was secured, particularly with the 40-, 60-, and 80-pound treatments. The heart rot of sugar beet was reduced greatly in 1941 by a 20-pound treatment after disease symptoms had appeared, and the heart rot was completely eliminated by heavier applications. It is also important to note that in 1941 substantial and significant increases in yield were

brought about by the delayed treatment. There was an increase of approximately 70 percent over the untreated plots in the 40-pound treatment. The decrease in the 60-pound treatment as compared with the 40-pound application was due principally to a reduction in stand caused by rhizoctonia root rot.

TABLE 8.—*The effect of a single dressing of borax during the growing season on the occurrence of internal black spot of Detroit Dark Red garden beets and of heart rot of sugar beets grown at Winneconne, Wis.*

Amount of borax applied (pounds per acre)	Detroit Dark Red garden beets				Sugar beets	
	1938 [1]		1939 [2]		1941 [3]	
	Plants diseased	Severity of disease	Plants diseased	Severity of disease	Heart rot	Yield per acre
	Percent	*Index*	*Percent*	*Index*	*Percent*	*Tons*
0	45.5	26.4	91.3	83.7	75.4	7.2
20	16.0	10.4	8.7	5.0	6.5	9.5
40	5.5	1.7	2.0	1.4	0	12.2
60			0	0	0	9.6
80	11.5	3.8	.7	.7		
Difference required for significance (19:1)	9.8	8.3				1.6

[1] Data based on 50-plant samples taken at random from each of 2 replicates.
[2] Data based on 50-plant samples taken at random from each of 3 replicates.
[3] Data based on two 50-foot rows from each of 4 replicates.

In 1939 at Winneconne a single midseason treatment was compared with treatments in which two and three applications were made at intervals of approximately 1 week. The results of this series are given in table 9. The disease was reduced to a small amount by a single 40-pound treatment. One supplementary 20-pound treatment reduced it still more while two subsequent 20-pound treatments completely eliminated black spot. This would indicate that the lack of complete control experienced in previous treatments at planting time or in midseason may be due either to lack of sufficiently complete distribution of boron in the soil or to lack of complete availability throughout the growing period. The greatest need for boron under average Wisconsin summer conditions for garden beets and sugar beets appears to be during the period immediately following the midpoint between sowing and harvest. In this experiment the first application of borax was made 44 days after sowing and 42 days before harvest.

TABLE 9.—*The effect of one, two, and three top dressings of borax during the growing season on the occurrence of internal black spot in Detroit Dark Red beets (Morse strain) sown at Winneconne, Wis., on June 20, 1939*

Amount of borax added per acre on dates indicated			Disease development [1]	
Aug. 3	Aug. 9	Aug. 16	Plants diseased	Severity of disease
Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
0	0	0	88.9	78.9
40	0	0	1.3	1.3
40	20	0	.6	.6
40	20	20	0	0

[1] Data from 50-plant samples taken at random from each of 3 replicates on September 14.

In 1940 and 1941 the method of midseason application, i.e., broadcast dry or applied as a spray in water suspension, was compared at Clyman with application at planting time. In 1940, since only a trace of black spot appeared, no data on disease control were secured. Yield records showed no consistent difference between the midseason spray treatment and side-dress treatment at planting at 20-, 40-, and 60-pound applications of borax. The midseason dry treatment showed a depression in yield at each level of borax when compared with the other two treatments. The differences were barely significant, however. The results in 1941 are given in table 10. The midseason spray was slightly better than the broadcast treatment at planting time in both disease control and in yield, while the midseason dry treatment was definitely less effective in both respects. It is therefore evident that midseason treatments are more effective if sufficient water is applied with the borax to facilitate immediate availability to the plants.

TABLE 10.—*Effect of borax applied by different methods on the incidence and severity of black spot and on yield of Perfected Detroit garden beets grown on Clyde silt loam at Clyman, Wis., 1941* [1]

Borax applied			Disease development		Yield per acre
Amount (pounds per acre)	Method	Time	Plants diseased [2]	Severity of disease [2]	
			Percent	*Disease index*	*Tons*
None			40	19.5	4.4
50	Broadcast	Planting time	5	1.2	4.6
50	Spray	Midseason	3	.9	5.0
50	Dry	Midseason	7	4.5	4.4

[1] Seed sown May 27; harvest data taken Aug. 19.
[2] Based on 25-beet samples from each of 3 replicates.

EFFECT OF APPLICATION OF BORAX ON CANNING QUALITY OF GARDEN BEETS

Samples were taken from the plots at Clyman for canning tests in 1938, 1939, and 1940 to determine whether boron applications had any detrimental effects on canning quality. In 1938 composite samples from the three replicates of each treatment were tested for the two varieties used. In 1939 and 1940 separate samples were taken from each replicate of plots receiving no borax, and those receiving 20, 40, 60, and 100 pounds per acre. No significant differences in quality were found between the samples from treated and untreated plots when examined by several canners who judged the canned product.

RELATION OF LIMING OF SOIL TO INTERNAL BLACK SPOT

The common occurrence of boron deficiency in garden beet in soils neutral or alkaline in reaction has already been noted. Overliming of soils has been associated with boron deficiency by numerous investigators. Presumably boron becomes less available to the plant in an alkaline soil, although the nature of its fixation is not fully understood. Cook and Millar (*8*), Muhr (*17*), Wolf (*26*), Ferguson and Wright (*13*), and Midgley and Dunklee (*16*) are of the opinion that boron is fixed in limed soils in the form of insoluble boron compounds, while Bobko

TABLE 11.—*The occurrence and extent of internal black spot in garden beets grown in 1940 on soils adjusted to various reaction levels after treatment with lime or sulfur in the spring of 1935*

Soil	Material applied in spring of 1935	Rate per acre	Soil reaction [1]							Internal black spot in—			
			Before treatment	1935	1936	1937	1938	1939	1940	Good For All		Detroit Dark Red	
										Plants diseased	Amount of disease	Plants diseased	Amount of disease
		Pounds	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*Percent*	*Index*	*Percent*	*Index*
Antigo silt loam	Sulfur	3,000	6.1	4.1	4.3	4.8	4.5	4.8	4.8	0	0	0	0
	do	2,000	6.0	4.4	4.8	5.4	5.4	5.3	5.3	0	0	0	0
	do	1,000	6.0	5.5	5.3	5.5	5.5	6.1	5.8	0	0	0	0
	None		6.1	6.0	6.0	5.8	6.3	6.2	6.3	0	0	0	0
	Calcium hydrate	3,000	6.3	6.8	6.2	6.4	6.7	7.6	6.5	0	0	0	0
	do	[2] 6,000	5.9	7.5	7.5	7.3	7.4	7.7	7.5	4	1	0	0
	do	[3] 9,000	6.1	8.0	7.8	7.5	7.9	7.8	8.0	40	27	2	1
Plainfield sand	Sulfur	700	5.0	4.5	4.6	4.1	4.5	4.5	4.3	(5)	(5)	(5)	(5)
	None		5.2	5.0	4.6	4.8	4.7	4.7	4.6	(5)	(5)	(5)	(5)
	Calcium carbonate	1,000	4.9	5.4	5.0	5.5	5.3	5.0	4.5	0	0	2	1
	do	3,000	4.9	5.3	5.7	6.0	5.4	5.5	4.9	14	7	6	3
	do	5,000	5.3	5.5	5.4	5.4	6.0	5.5	5.2	26	24	4	2
	do	7,000	5.2	6.0	6.5	6.6	6.5	6.5	6.3	22	12	6	1
	do	[4] 9,000	5.3	6.1	6.5	7.0	6.8	7.0	6.7	40	27	4	1

[1] Except for the sampling in the spring of 1935 before treatment, the reaction tests were made from samples collected in September of each year. Reaction tests were made by Dr. Harold H. Hull, Department of Soils.
[2] Plus 1,000 pounds calcium hydrate added in spring of 1936.
[3] Plus 2,000 pounds sodium carbonate added in spring of 1936.
[4] Plus 3,000 pounds calcium carbonate added in spring of 1936.
[5] No plants developed beyond the seedling stage.

et al. (*4*), Naftel (*18*), and Hanna and Purvis (*14*) are of the opinion that activity of soil micro-organisms accounts for some of the fixation.

In 1940 a study was made of the development of internal black spot in Good For All and Detroit Dark Red (Ferry strain) beets on two series of plots which had been treated with various amounts of lime or sulfur in the spring of 1935 to adjust the soil reaction to various levels. One series was located on Plainfield sand in central Wisconsin (near Arnott, Portage County); the other was on Antigo silt loam in northcentral Wisconsin (near Antigo, Langlade County). Each of the series was designed to provide a range of reactions from about pH 4.5 to 8.0. It may be seen from the annual pH readings in table 11 just how the reactions became adjusted and the extent to which they reverted to the original level during the 5-year period. Beets planted on these plots in the sixth season after treatment (1940) were examined for black spot (table 11).

In Antigo silt loam no black spot was found except in those treatments in which the soil reaction was still alkaline, and in the more susceptible of the two varieties the disease was decidedly more severe where the heavier treatment with lime had maintained the highest alkalinity. In Plainfield sand, however, the disease was marked at the 3,000-pound treatment, increasing with heavier dosages of lime. This correlation prevailed in spite of the fact that at no time did the soil reaction reach neutrality except in the heaviest treatment and that in two treatments the reaction had receded to around pH 5.5 at least 2 years before that in which beets were grown. It seems quite evident that in this light soil the liming treatment tied up boron without neutralizing the soil solution and that some of the boron remained unavailable to plants over a period of several years. This soil was so low in calcium that beets grew very poorly in the untreated and sulfur-treated plots, which were extremely acid, while the 1,000-pound-lime-treated plot, although its reaction was equally acid, supported good growth. This raises the question whether borax should not be applied generally on this soil in order to offset the fixation of available boron when it is limed for legumes or other crops.

DISCUSSION

The occurrence of internal black spot in garden beets is much more important than the relative percentage of affected plants would signify. This is because the necrotic areas are detected with difficulty and because their presence in the canned product even in small amounts has been ruled by food inspection authorities as sufficient to render a given lot unfit for sale as human food. Thus, a relatively small amount is enough to cause a complete loss. The initial purpose in the study with garden beets was to determine the relation of boron to the development of internal black spot, to learn whether any other minor element had a supplementary influence, and to ascertain how effective applications of borax to deficient soils could be for the control of the disease.

From the results presented in this paper there is abundant evidence that boron deficiency in the soil is responsible for the nonparasitic abnormal development of the garden beet which leads to necrosis in the root. It is also shown that varieties of garden beet vary in their pathologic response to this deficiency. Furthermore, table beets are in general more susceptible to this disease than sugar beets.

In the experiments conducted, the application of borax to the soil, either as a side-of-row band or broadcast, has never failed to reduce the amount of disease. However, as a rule, increasing amounts of borax, although they have tended to reduce the amount of black spot, have failed to control the disease completely. When substantial amounts of manganese, zinc, copper, cobalt, and sodium were applied no consistent change in the extent of black spot resulted. This seems to remove the probability of black spot being associated with a deficiency of other trace elements now regarded as essential at some time or other in the normal development of most crop plants.

It has become apparent in the studies in sand culture, in pot cultures with soil, and in midseason applications of borax in the field that boron when first applied as borax or boric acid is readily available through roots or leaves and is transported rapidly to those meristematic regions where its deficit becomes most quickly apparent. It is equally apparent that boron rather readily becomes locked in an immobile form in the tissues of maturing organs or regions within such organs, and once in that state it is of little immediate use to the meristematic tissues. This observation is in agreement with the recent work of Wolf (*26*) with cauliflower.

Since the quality requirements of table beets make it important that meristematic regions in the root have adequate boron at all times, the program of control must take into account the availability of boron in the soil and its mobility in the plant. The consistent lack of complete control when large quantities of borax were applied at planting time has not been adequately explained. It is apparent from 4 years' observation that the disease develops in late July or August in Wisconsin, when the crop is usually about midway between sowing and harvest. At this period 40 to 60 days have elapsed after the application of borax. When borax was applied in midseason before the appearance of symptoms the disease was controlled as well as and in some cases better than when applied at the time of planting.

In the experiments on silty clay loam at Winneconne about the same degree of control was secured in the second year as in the season immediately following spring application, indicating that a considerable part of the boron applied remained available in this alkaline soil in addition to that which may have leached away or become fixed chemically or biologically. On the other hand, it was obvious from the experiments on sandy loam at Arnott that treatments with lime tended to result in less available boron 5 years later and long after the soil reaction had become quite acid.

The most acute and severe development of black spot has occurred when a protracted dry spell during which the plants have slowed down very decidedly in growth is followed by abundant soil moisture and a sudden increase in growth. Under such conditions the plants seem to be unable to get adequate boron for all their needs and necrotic break-down occurs. If borax has been applied to the soil most plants secure enough boron, but some fail to do so and a certain amount of disease results. Some indication of this may be seen in the work of Eaton and Wilcox (*12*) on boron toxicity in soils of various types under various conditions. They point out that—

When new boron is added to a soil * * * drying is an important adjunct to fixation. When soils are dried, the concentrations of all soil-solution constituents are increased to saturation, which is followed by precipitation. In the soils with-

out added boron this precipitation is reversible, since on rewetting, the initia concentrations are again found. When boron is added to the soil and then dried, however, a part of that which has been added is not recovered on resuspension.

Variation between individual plants and between strains of garden beets is another factor which probably enters into the clinical picture of the black spot disease. It has been shown that Good For All is very susceptible and that the two strains of Detroit Dark Red differ in their reaction under some field conditions. It would be of interest to inquire further into the nature of these differences. The nature and extent of the root system might have an important bearing upon the reaction of individuals or strains in the acquisition of boron from the soil when it is needed most vitally by the plant.

SUMMARY

The development of the symptoms of boron deficiency on young seedlings of garden beet in quartz-sand culture is described. The disease appeared promptly as an intensification of red pigment, unilateral development of leaf lamina, stunting, and death of the growing point. In plants allowed to grow slightly older before boron deficiency became acute, symptoms of the same type appeared more slowly. Boron became fixed promptly in the tissues, with the result that mature leaves did not yield mobile boron readily to meristematic regions. However, soluble boron as boric acid in the nutrient supplied through the roots or applied to older leaves was transported readily to the aerial growing tip, stimulating normal growth. In garden beets grown in complete solution long enough for taproot enlargement, typical black spot appeared gradually after the boron supply was eliminated, in rings in which bundles had differentiated most recently. Plants grown in the greenhouse on soil from fields where severe black spot of garden beet or heart rot of sugar beet had occurred ordinarily did not show acute or severe symptoms, although growth responses to the addition of borax to the soil were secured. Greenhouse diagnosis of boron deficiency by pot tests was not satisfactory.

In a survey of the occurrence of internal black spot of garden beet in Wisconsin during the growing seasons of 1937, 1938, 1939, and 1940, the disease was found most often in soils with neutral or alkaline reaction. It occurred on soils ranging from light sandy loam to dark silty clay loam and was about equally prevalent in high and low parts of the field.

In field experiments at several locations in Wisconsin during the seasons of 1938, 1939, 1940, and 1941 the application of borax to the soil consistently reduced the internal black spot disease of garden beet and the heart rot disease of sugar beet.

Other minor elements, including manganese, zinc, copper, iron, and cobalt, when applied as soluble salts, did not reduce the disease.

Heart rot of sugar beet was corrected effectively with smaller applications of borax than were necessary for the control of internal black spot of garden beet. Even as heavy applications as 100 pounds of borax per acre often did not completely eliminate the black spot disease.

Broadcast applications on slightly alkaline Poygan silty clay loam in 1938 were effective for two successive seasons and partly so for the third season. During the first season broadcast applications of as

high as 60 pounds per acre, disked into this soil prior to planting, were not injurious to either garden or sugar beet and only very slightly so to the garden bean, which is very sensitive to boron toxicity.

Applications of borax with the fertilizer in bands 1½ to 2 inches removed from and at the same depth as the seed were equal in effectiveness to broadcast treatments.

Increases in yield of garden beet commonly, but not always, resulted from borax applications. An increase occurred in one instance where the boron naturally available in the soil was sufficient to prevent black spot.

Midseason applications of borax, either in the dry form or in liquid suspension, were effective in controlling black spot when carried out just before or at the time of the first appearance of symptoms in the leaves.

Applications of borax up to 100 pounds per acre did not alter the canning quality of the garden beet.

Boron deficiency appeared in garden beets grown on Antigo silt loam limed 5 years earlier to maintain an alkaline reaction during the intervening period. Where the lime applied had not brought the reaction up to neutrality black spot did not occur. In Plainfield sand similarly treated, however, black spot appeared in all limed plots and severely in one in which a pH reading as high as 6.0 had been recorded in only 1 of the intervening 5 years. This shows that soil types differed in the degree to which available boron was tied up by liming and that, in sandy soil, boron deficiency was made acute by liming without the soil solution approaching an alkaline reaction and for a 5-year period after the treatment.

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THE NUTRITIVE VALUE OF CERTAIN FISH MEALS AS DETERMINED IN TESTS WITH SWINE AND RATS[1]

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INTRODUCTION

The use of fish meal in mixed protein supplements for fattening swine has become widespread in certain sections of the Corn Belt. The value of this practice was demonstrated by Vestal[2] at the Purdue Experiment Station.

As several different kinds of fish meals are available, there is a demand for information on their comparative feeding value. Menhaden meal from the east coast and sardine and herring meals from the west coast are the most readily available at prices the swine feeder is willing to pay.

The purpose of this investigation was to determine whether there is any significant difference in the nutritive value of several commercial fish meals when fed to swine and rats.

LITERATURE REVIEW

Numerous investigations have been conducted with chickens and white rats which show that the nutritive value of fish meals may vary as a result of source of material and method of processing. It has repeatedly been demonstrated (*1, 2, 3, 5, 10, 11, 12, 13, 14, 16, 17, 18*)[3] that vacuum-dried meals and meals dried at reduced temperatures are superior in feeding value to those dried at high temperatures. White fish meals have frequently given better results than dark fish meals, although the variety of fish from which the meal is made appears to have much less effect on the finished product than the freshness of the material and the method of processing.

The temperature used in the drying process is one of the most important causes of variation in the finished product. The consistently high feeding value reported for vacuum-dried meals as compared to flame-dried meals indicates the necessity of controlled temperature during the drying process. Ingvaldsen (*6*) found that when meals were subjected to temperatures higher than 190° C., a diminution in arginine and cystine occurred. He also reported (*7*) lowered tyrosine, tryptophane, and cystine content of fish meals due to putrefaction.

Few experiments to determine the comparative value of different kinds of fish meal in swine rations have been reported. Investigators at the Iowa Agricultural Experiment Station (*8*), Foster and Hostetler (*4*), Kyzer and Jones (*9*), and Willman and Morrison (*19*) found very

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[2] See Vestal, C. M. mimeographed reports, 1937, 1938, 1939, 1940.

[3] Italic numbers in parentheses refer to Literature Cited, p. 133.

little difference in the feeding value of different fish meals for fattening swine, particularly when the meals were fed in combination with other protein feeds.

MATERIALS AND METHODS

Feeding trials were conducted with both swine and white rats. Swine were used for comparative trials of the feeding value of flame-dried menhaden, sardine, and herring fish meal in a commonly used mixed protein supplement. Rats were used to determine the protein efficiency of the fish meals when they were fed as the only protein supplement to corn. Two vacuum-dried whitefish meals and a steam-dried sardine meal were also tested under these conditions. The rate and efficiency of gain were used as a basis for evaluating the results. The results were treated statistically by the analysis of variance technique as given by Snedecor (*15*). Significant mean differences at the 5-percent level and highly significant mean differences at the 1-percent level are given when such differences existed.

SWINE-FEEDING TRIALS

Three similar feeding trials with the fish meals were carried out with swine in dry lot. The first trial was conducted during the winter of 1939–40, the second during the summer of 1940, and the third during the winter of 1940–41.

EXPERIMENTAL ANIMALS AND RATIONS

The pigs were obtained from the Purdue experimental swine herd of Duroc-Jerseys. All the animals were healthy and normal in size for their age at the beginning of the experiment. In each trial the pigs were divided as uniformly as possible according to size, sex, and probable ability to gain in the feed lot. The average weight of the pigs was approximately 70 pounds each at the beginning of the experiment, and they were fed until they reached a market weight of approximately 225 pounds. Ten pigs were started in each lot in the first trial, 15 in the second, and 12 in the third. One pig in the lot, fed sardine meal, died in the second trial, and 1 pig was removed from the sardine feed-lot in the third trial after 69 days. The quarters for each lot of pigs consisted of a colony house and a dry lot approximately 30 feet wide and 60 feet deep.

The rations were composed of No. 2 yellow shelled corn fed free choice with a protein supplemental mixture composed of 40 percent expeller-process soybean oil meal, 20 percent meat and bone scraps, 20 percent fish meal, 10 percent cottonseed meal, and 10 percent alfalfa leaf meal. A mineral mixture containing 10 pounds of steamed bonemeal, 10 pounds of pulverized limestone, and 1 pound of common salt was available to the hogs at all times. Block salt was also kept before the hogs. The shelled corn, mixed protein supplement, mineral mixture, and salt were fed free choice in self-feeders.

The experimental rations differed only in the kind of fish meal included in the mixed protein supplement. The other constituents of the protein supplement were obtained on the local market. A complete chemical analysis was made of each ingredient of the mixed supplement (table 1).

TABLE 1.—*Analysis of the feeds used in the mixed protein supplement of the swine rations*

Ingredient	Trial No.	Moisture	Protein	Fat	Fiber	Ash	Nitrogen-free extract
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Menhaden fish meal (flame-dried)	1	5.7	66.2	4.4	0.3	17.5	5.9
	2 and 3	6.4	65.8	5.1	.5	18.3	3.9
Sardine fish meal (flame-dried)	1 and 2	5.1	70.9	5.5	.4	11.4	6.7
	3	6.7	65.1	4.3	.8	16.9	6.2
Herring fish meal (flame-dried)	1 and 2	5.1	75.1	6.6	.4	11.1	1.7
	3	8.2	69.9	9.2	.8	11.5	.4
Soybean oil meal	1	7.7	45.4	6.8	5.6	5.0	29.5
	2 and 3	8.7	46.1	5.5	6.1	4.7	28.9
Meat and bone scraps	1	5.0	51.0	7.6	2.4	30.1	3.9
	2 and 3	5.7	49.9	6.2	2.0	34.2	2.0
Cottonseed meal	1	6.2	41.1	6.2	9.9	6.3	30.3
	2 and 3	4.8	41.3	5.8	10.0	6.9	31.2
Alfalfa leaf meal	1	6.8	18.2	2.9	20.4	9.1	42.6
	2 and 3	4.3	24.9	3.6	16.2	8.2	42.8

The initial weight recorded for each pig is the average of weights taken on 3 consecutive days. The 3-day average weight was considered the starting weight. A similar procedure was followed in determining the final weight at the close of the experiments. Group weights were taken every 10 days and individual weights every 30 days. The feed records were tabulated by 10-day periods corresponding to the 10-day weight periods.

EXPERIMENTAL RESULTS

Because of the difference in protetin content of the fish meals there was a slight variation in the total protein of the supplements containing the different fish meals. This variation was not more than 2.4 percent in any of the trials.

The results of the three feeding trials are shown in table 2. The

TABLE 2.—*Weights, gains, and feed consumption of pigs fed different kinds of fish meal* [1]

[Summary of 3 trials]

Fish meal in ration	Trial No.	Hogs fed	Period on feed	Average initial weight	Average final weight	Average total gain	Average daily gain	Average daily feed consumption	Total feed per 100 pounds of gain	Ratio of consumption of protein supplement to corn
		Number	*Days*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
Menhaden (flame-dried).	1	10	90	71.1	222.7	151.6	1.68	6.6	383	1:7.9
	2	15	85	69.0	205.0	136.0	1.60	5.9	368	1:6.0
	3	12	100	57.8	233.3	175.5	1.76	7.1	400	1:9.5
	Average	12.3	91.7	66.0	219.0	153.0	1.67	6.4	384	1:7.6
Sardine (flame-dried).	1	10	90	71.0	228.7	157.7	1.75	6.4	364	1:5.4
	2	14	85	69.0	205.4	136.4	1.60	5.9	366	1:7.7
	3	11	100	58.2	238.4	180.2	1.80	7.1	396	1:10.3
	Average	11.7	91.7	66.1	222.4	156.3	1.71	6.4	376	1:7.6
Herring (flame-dried).	1	10	90	71.5	215.2	143.7	1.60	6.3	393	1:5.8
	2	15	85	69.0	206.5	137.5	1.62	6.0	370	1:7.2
	3	12	100	57.9	230.8	172.9	1.73	6.8	394	1:10.2
	Average	12.3	91.7	66.1	216.8	150.7	1.64	6.3	385	1:7.6

[1] Rations self-fed, free choice: Corn, fish-meal supplement mixture, and mineral.

differences in gains between the lots were not statistically significant in any of the trials.

In trial 1, conducted during the winter of 1939–40, the rate of gain was 1.68, 1.75, and 1.60 pounds respectively for the menhaden-, sardine-, and herring-fed lots. The group receiving herring meal required 392.7 pounds of feed for each 100 pounds of gain, while the lot fed menhaden meal required 383.3 pounds and the lot fed sardine meal required only 363.9 pounds. The lots fed sardine and herring meal both consumed excessive amounts of the protein supplement.

In trial 2, conducted in the summer of 1940, for all three lots the rates of gain and of feed consumption for each 100 pounds of gain were practically the same; however, in this trial the lot receiving the menhaden meal consumed more supplement than the lots receiving sardine and herring meals. This may have been due to the fact that a fresh supply of menhaden meal was used in this trial.

The third trial, conducted in the winter of 1940–41, confirmed the results of the previous trials. There was very little difference in either rate of gain or feed consumption for the three lots.

SUMMARY OF RESULTS

The results with swine indicate that the beneficial qualities of fish meal as a constituent of mixed supplements were supplied equally well by menhaden, sardine, and herring meals. However, there was a slight tendency for the gains to be a trifle higher on the sardine fish-meal ration and the feed required for 100 pounds gain was also slightly less. The results also show that the same commercial brand of fish meal may vary considerably in palatability as judged by a comparison of feed consumption.

RAT—FEEDING TRIALS

The feeding experiments with rats consisted of two parts. The first was designed to gain information on a larger number of fish meals than was studied with hogs. The consumption of feed was unrestricted and fish meal was used as the only protein supplement to corn. The second was planned to obtain a more accurate comparison of the protein efficiency of the fish meals used in the swine-feeding trials by equalized feeding of groups of three animals.

FEED CONSUMPTION UNRESTRICTED

In the "ad libitum" experiments, six different fish meals were compared in two similar trials. The mixed rations consisted of finely ground yellow corn with sufficient fish meal added to bring the protein content to approximately 14 percent. One percent of cod-liver oil and 2 percent of a mineral mixture were included in the rations. The composition of the rations is shown in table 3 and the analysis of the fish meals in table 4.

TABLE 3.—*Rations fed to rats to determine the comparative value of different fish meals*[1]

Feed used	Ration number					
	1	2	3	4	5	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Yellow corn	87.2	87.2	87.2	87.2	87.2	86.5
Menhaden fish meal (flame-dried)	9.1					
Sardine fish meal (flame-dried)		8.5				
Sardine fish meal (steam-dried)			8.5			
Herring fish meal (flame-dried)				8.0		
Whitefish meal (vacuum-dried)					9.3	
Whitefish meal blend (vacuum-dried)						10.5
Dextrin	.7	1.3	1.3	1.8	.5	0.0
Mineral [2]	2.0	2.0	2.0	2.0	2.0	2.0
Cod-liver oil	1.0	1.0	1.0	1.0	1.0	1.0
Total protein	14.03	14.10	14.05	13.91	14.02	13.89

[1] Feed consumption unrestricted.
[2] The mineral mixture was composed of 10 parts limestone, 10 parts special steamed bonemeal, and 1 part common salt.

TABLE 4.—*Analysis of fish meals used in rat experiments*

Fish meal in ration	Moisture	Protein	Fat	Fiber	Nitrogen-free extract	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Menhaden (flame-dried)	5.7	66.2	4.4	0.3	5.9	17.5
Sardine (flame-dried)	5.1	70.9	5.5	.4	6.7	11.4
Sardine (steam-dried)	5.5	71.0	3.4	.6	3.8	15.7
Herring (flame-dried)	5.1	75.1	6.6	.4	1.7	11.1
Whitefish meal (vacuum-dried)	13.4	64.1	3.6	.4	0	20.9
Whitefish meal blend (vacuum-dried)	6.5	57.2	7.5	2.2	3.2	23.4

Corn supplied 57.9 percent of the total protein in the ration and fish meal 42.3 percent. When necessary, dextrin was added to balance the ingredients. The corn used was No. 2 yellow dent, and the fish meals were standard commercial grades.

Albino rats were started on feed in individual cages at about 3 weeks of age, when they weighed 35 to 45 gm. each, and were fed for a period of 8 weeks. Weekly records were kept of the gains made and the feed consumed. Ten litters of rats were used, one animal from each litter being represented on each ration. In the first trial there were six males and four females on each ration.

The results of the two trials are shown in table 5. The differences in gain in the first trial were not significant; however, there was a

TABLE 5.—*The nutritive value of different fish meals in rations for rats* [1]

Fish meal in ration	Trial No.	Mean gain	Adjusted mean gain	Mean feed consumption	Feed per gram of gain
		Grams	*Grams*	*Grams*	*Grams*
Menhaden (flame-dried)	1	185.9	199.1	777.9	4.19
	2	192.6	200.1	715.9	3.72
Sardine (flame-dried)	1	211.6	197.9	851.3	4.02
	2	243.2	211.5	826.8	3.40
Sardine (steam-dried)	1	188.5	197.3	788.6	4.18
	2	208.3	199.3	762.7	3.66
Herring (flame-dried)	1	177.7	204.4	741.2	4.17
	2	170.5	215.4	610.0	3.58
Whitefish meal (vacuum-dried)	1	198.8	185.0	851.7	4.28
	2	205.3	191.8	775.2	3.78
Whitefish meal blend (vacuum-dried)	1	196.2	172.8	879.7	4.48
	2	190.0	191.8	732.1	3.85

[1] Feed consumption unrestricted.

significant difference in the amount of feed consumed, indicating that the fish meals varied in palatability. In order to adjust the mean gains to the same level of food consumption the data were analyzed by the analysis of covariance (table 5). A difference in the adjusted mean gain of 15 gm. is significant at the 5-percent level and a difference of 20 gm. is significant at the 1-percent level. The following significant differences in the nutritive value of the fish meals are shown: Herring higher than whitefish and whitefish blend; sardine higher than whitefish blend; sardine (steam-dried) higher than whitefish blend; and menhaden higher than whitefish blend.

In the second trial eight males and two females were fed on each ration. As in the previous trial each rat had a litter mate on every other ration.

The analysis of the data of the second trial shows there was a significant difference in the gains made as well as in the amount of feed consumed. The least significant mean differences were 32.8 gm. for the mean gain and 83.6 gm. for the mean feed consumption. The data show that the flame-dried sardine, steam-dried sardine, and whitefish meal all produced significantly larger gains than the herring meal. However, the feed consumption for the herring meal group was significantly lower than for any other ration. This may have been due in part to its high fat content.

In order to determine whether the differences in gain resulted entirely from variation in quantity of feed consumed or from a difference in the nutritive value of the fish meals, the mean gains were adjusted for feed consumption as in trial 1. The results (table 5) demonstrate that palatability does not account for all the difference in gain. A difference of 14.5 gm. gain is significant at the 5-percent level and a difference of 19.4 gm. is significant at the 1-percent level.

An interesting result revealed by this analysis was that the herring meal was apparently the least palatable and produced the smallest gain, yet it appeared to have a higher nutritive value than some of the other fish meals. The results of the controlled feeding trails substantiate this conclusion.

CONTROLLED FEEDING

The menhaden, sardine, and herring fish meals were compared by applying the paired feeding technique to triplicates. Each member of a triplicate received the same amount of feed, the member consuming the smallest amount limiting the intake of the other two. A triplicate was composed of litter mates of the same sex that did not vary more than 3 gm. in starting weight.

These trials were conducted with rations of approximately 8.5, 10.5, and 12.5 percent protein (table 6). The proportion of protein supplied by the corn and fish meal was kept the same at each level. The feed was weighed out to each rat daily to the nearest 0.1 gm. on a torsion balance and the gain in weight of the rats was recorded by 7-day periods. Eight rats were fed on each fish meal at each protein level for a period of 7 weeks.

A summary of the results for the three protein levels is shown in table 7. The least significant mean differences in gain were 3.85, 3.47, and 4.01 for the 12.5-, 10.5-, and 8.5-percent levels of protein respectively. The data indicate that there were significant differ-

ences in the nutritive value of the meals. Except at the 8.5-percent protein level the sardine meal showed a higher nutritive value than the herring and menhaden meals, and the herring meal showed a higher nutritive value in all three trials than the menhaden. At none of the levels of protein intake was the difference in the gains made on the sardine and herring meals significant at the 1-percent level. However, at all three levels of protein intake, both the sardine and herring meals produced gains that were significant at the 1-percent level over those produced by the menhaden meal. This is also shown by the means of the three levels for each fish meal (table 7, last column) where a difference of 2.47 gm. is necessary for the 5-percent level and a difference of 3.31 gm. for the 1-percent level.

TABLE 6.—*Rations fed to rats in controlled feeding tests of different fish meal rations*

Feed used	Trial 1 rations (at the 12.5-percent protein level)			Trial 2 rations (at the 10.5-percent protein level)			Trial 3 rations (at the 8.5-percent protein level)		
	1	2	3	1	2	3	1	2	3
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Precent*
Yellow corn	80.0	80.0	80.0	67.6	67.6	67.6	55.3	55.3	55.3
Menhaden fish meal	8.3			7.1			5.8		
Sardine fish meal		7.7			6.6			5.4	
Herring fish meal			7.3			6.2			5.1
Dextrin	8.7	9.3	9.7	22.3	22.8	23.2	35.9	36.3	36.6
Mineral	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cod-liver oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total protein	12.31	12.44	12.24	10.72	10.60	10.59	8.63	8.61	8.66

TABLE 7.—*The nutritive value of different fish meals in rat rations at three levels of protein intake* [1]

Fish meal in ration	Rats at each protein level	At the 12.5-percent protein level				At the 10.5-percent protein level				At the 8.5-percent protein level				Mean gain on each fish meal [2]
		Average initial weight	Average final weight	Average total gain	Average feed consumed	Average initial weight	Average final weight	Average total gain	Average feed consumed	Average initial weight	Average final weight	Average total gain	Average feed consumed	
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Menhaden	8	37.50	149.50	112.00	414	35.75	145.50	109.75	436	36.10	88.35	52.25	290	91.33
Sardine	8	37.75	161.87	124.12	414	35.50	153.75	118.25	436	36.00	92.12	56.12	290	99.50
Herring	8	37.75	157.37	119.62	414	35.75	149.75	114.00	436	36.10	94.10	58.00	290	97.21

[1] Feed consumption restricted.
[2] Least significant difference at the 5-percent level-2.472 gm.; least significant difference at the 1-percent level-3.307 gm.

The data obtained in this experiment in which feed consumption was controlled confirm the results shown by the analysis of covariance in the ad libitum feeding trials; namely, that the herring meal had a nutritive value nearly as high as the sardine meal although it was much less palatable. In the paired feeding trials the rats receiving herring meal most consistently limited the feed intake of the other members of the triplicate. The menhaden meal was second in this respect.

DISCUSSION OF RESULTS

The rat-feeding experiments showed that the fish meals differed in palatability and nutritive value. However, these differences were not detectable in swine-feeding trials when the fish meals were fed in combination with several sources of protein.

The whitefish meals (vacuum-dried) used in this investigation were not found to be superior to flame-dried menhaden meal as has been reported in the literature (*3, 12, 14, 16*).

It is realized that additional work in this field of investigation is necessary before definite conclusions can be formed, for the same kind of fish meal may vary considerably from time to time because of uncontrollable conditions inherent in the fish-meal industry. The results of this investigation should therefore not be applied to all commercial fish meals until they are verified by testing a larger number of samples of the same kind of meals.

SUMMARY AND CONCLUSIONS

A study was made of the comparative nutritive value of several different commercial fish meals by including them in rations of swine and rats.

Menhaden, sardine, and herring fish meals were found to have equal value in a mixed protein supplement when fed to swine.

The experiments with rats showed differences in palatability and nutritive value of the fish meals when they were fed as the only protein supplement to corn.

Ad libitum feeding experiments with rats showed that the sardine and whitefish meals were more palatable than the menhaden and herring meals. Sardine meal appeared to be slightly superior to the other meals in palatability and nutritive value. Although herring meal was the least palatable it had a nutritive value comparable to sardine meal.

Controlled feeding trials with rats showed that the sardine and herring meals were superior in nutritive value to the menhaden meal.

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FUNGI ASSOCIATED WITH CERTAIN AMBROSIA BEETLES [1]

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INTRODUCTION

The association of certain fungi wth the tunnels of ambrosia beetles has been recognized for nearly 100 years (*1*).[3] These ambrosia fungi apparently are used as food by the beetles, and different fungi are cultivated by different species or groups of species (*2*). Recently, Leach et al. (*3*), in a paper on two ambrosia beetles and their associated fungi, summarized the past work on ambrosia fungi, pointing out particularly the scarcity of exact information on this group.

In 1937 the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, started a general study of the biology of ambrosia beetles of the Southern States, and the Division of Forest Pathology cooperated in studying the fungi associated with certain ambrosia beetles of economic importance in the deterioration of green hardwood logs and lumber. The present paper is a report of the mycological findings of this study.

MATERIALS AND METHODS

Wood infested with ambrosia beetles was furnished largely by H. R. Johnston, of the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, at Saucier, Miss. Some material was gathered at various sawmills in Louisiana. All the beetle material was identified by Johnston.

All cultural studies were made on malt agar (1½ percent agar and 2½ percent malt) at room temperature, approximately 75° to 85° F. Isolations were made by allowing live beetles to walk on the agar surface and also by making transplants from the wood adjacent to tunnels. Some cultures were purified by making single-spore transfers from the original cultures secured from the beetles or from wood. Numerous freehand and microtome sections were cut from wood blocks that contained insect tunnels, for the study of the fungi as they occur in nature.

The species of ambrosia beetles studied were *Platypus compositus* Say, *Pterocyclon mali* (Fitch), *P. fasciatum* (Say), *Xyleborus affinis* Eich., and *X. pecanis* Hopkins.

[1] Received for publication April 22, 1942. The Southern Forest Experiment Station of the Forest Service, U. S. Department of Agriculture, cooperated in these investigations by providing facilities for field studies at Saucier, Miss.

[2] The author is indebted to Ross W. Davidson, of the Division of Forest Pathology, for advice on the taxonomy of the fungi described; and to Edith K. Cash, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, for translating descriptions of species into Latin.

[3] Italic numbers in parentheses refer to Literature Cited, p. 144.

FUNGI ISOLATED FROM AMBROSIA BEETLES

WOOD-STAINING FUNGI

Although a great many fungi, bacteria, yeasts, and nematodes were isolated from ambrosia beetles and their tunnels, only the food fungi and general-staining fungi were given special attention. Stain accompanying ambrosia beetle attack is of two distinct types: (1) A restricted darkening adjacent to the tunnels, and (2) general staining of the type common in sapwood without beetle attack.

Restricted blackened areas are common, if not universal, around tunnels of *Platypus compositus*, *Pterocyclon mali*, and *P. fasciatum*

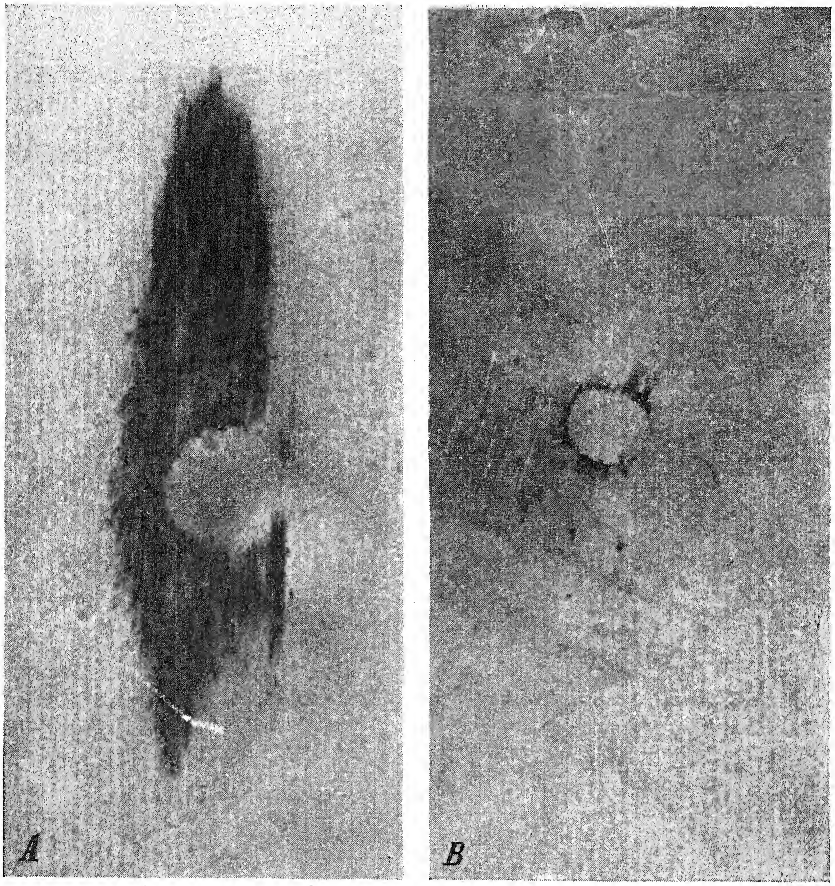

FIGURE 1.—Black stain around tunnels of two ambrosia beetles: *A*, *Platypus compositus*; *B*, *Pterocyclon mali*. × 15.

in sapwood but absent or indistinct in heartwood. Stain is commonly absent around the tunnels of *Xyleborus affinis*, but occasionally a light-brown stain is found around the tunnels of this species in sweetgum (*Liquidambar styraciflua* L.) and southern sweetbay (*Magnolia virginiana* L.). Around the tunnels of *Xyleborus pecanis* no black stain was observed, although some browning of the adjacent wood cells occurred. Black stain is most pronounced with *Platypus*

compositus (fig. 1, *A*), extending longitudinally from the tunnel as much as 5 mm., although laterally it spreads but a few cells from the edge of the tunnel. With *Pterocyclon* (fig. 1, *B*) the black is just as intense but usually extends less than 1 mm. from the tunnel.

Isolations from black stain around tunnels yielded a number of organisms, but the only ones consistently isolated were the ambrosia or food fungi of the beetles concerned; these fungi are described later in this paper. Microscopic examinations of the blackened areas showed the wood cells to be filled with fungus material of a deep-brown color, which in some cases could be identified as an ambrosia fungus by the presence of spores. When the surface of sapwood blocks of sweetgum and oak was sterilized for 30 seconds in boiling water, and the blocks were then inoculated with cultures of the ambrosia fungi, stain was produced. This stain, however, was not as intense as that found around the tunnels of *Platypus compositus* and *Pterocyclon* spp. Indications are that the stain around beetle tunnels is caused by the ambrosia fungi, although the color may be intensified by reactions of these fungi with insect secretions or other organisms, such as yeasts and bacteria, which are commonly present.

General staining of the type common in sapwood without beetle attack is not universal with ambrosa beetle attack. However, past tests and observations have shown that severe staining of logs is not prevented by chemical sprays when ambrosia beetle attack is heavy (*5*). The role of ambrosia beetles in disseminating general wood-staining fungi has been discussed more fully in another paper (*7*).

AMBROSIA OR FOOD FUNGI

AMBROSIA FUNGUS OF PLATYPUS COMPOSITUS

The only fungus consistently observed in the tunnels of *Platypus compositus* or consistently isolated from the adults or wood adjacent to the tunnels of this insect was a species of *Endomyces* Rees. A review of the literature disclosed no described species having the spore, asci, and hyphae measurements of this ambrosia fungus or any species of *Endomyces* with only two-spored asci. Therefore, the fungus is here described as a new species:

Endomyces bispora sp. nov.

On malt agar, colonies (fig. 2, *C*) are very slow growing, reaching 5–8 mm in radius in 6 days at room temperature (75° to 85° F.). At first the colony is hyaline, then turns a light yellowish brown, although under the microscope the hyphae and spores appear hyaline. The edge of the colony is distinctly mycelial with many conidia formed singly or in heads on short recumbent conidiophores or laterally on the hyphae (fig. 2, *A* and *B*). The cells of the conidiophores often disjoint and act as conidia, and buds form from the conidia. Most of the colony is composed of a slimy mass of conidia and ascospores, which give it a yeastlike appearance. Practically no aerial mycelium is formed.

Conidia commonly germinate on malt agar by producing buds, forming a yeastlike colony from the margin of which hyphae eventually protrude. No germination of ascospores was observed.

Conidia, including disjointed conidiophores and buds, are hyaline, unicellular, and of various shapes and sizes: 2.5μ–$12.5\mu \times 2.2\mu$–5.0μ, averaging $6.3\mu \times 3.8\mu$, usually elongate and somewhat pear-shaped. Asci are hyaline, slightly pear-shaped, and are usually formed terminally in small groups of two to six. Asci are occasionally formed singly at the tip or nodes of the asci-bearing hyphae (fig. 2, *A*) or in compound heads on short branches. Asci are 7.5μ–$11.2\mu \times 6.2\mu$–10.0μ, averaging 7.4μ wide and 8.6μ long. Asci-bearing hyphae range from 1.8μ to 3.3μ in diameter, averaging 2.5μ. Ascospores (fig. 2, *A*) are hyaline, hat-shaped, 6.2μ–9.8μ, averaging 7.2μ, across the brim, and 1.8μ–3.2μ, averaging 2.4μ,

are usually alined within the ascus with the brims extending from the base to the in width perpendicular to the brim. The brim surface is concave. The spores

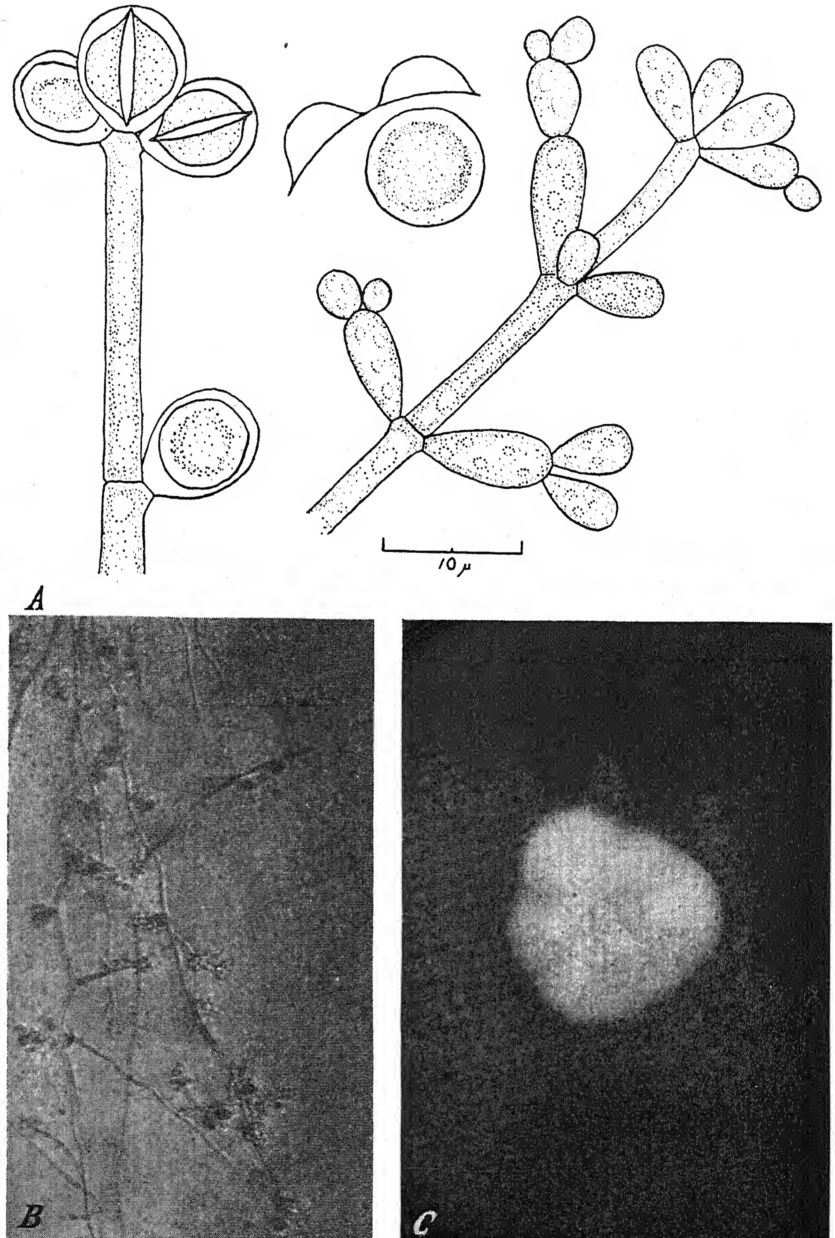

FIGURE 2.—*Endomyces bispora* on malt agar: *A*, Asci, ascospores, and conidia; *B*, margin of a culture showing hyphae and conidia production, × 150; *C*, culture 15 days old grown at about 80° F., × 1.

tip of the ascus and nearly filling the ascus. Only two spores were observed in each ascus.

Isolated from adults of *Platypus compositus* Say and from wood adjacent to their tunnels. Observed in pecan (*Carya* sp.), sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and locust (*Gleditsia* sp.).

Culturae in agaro maltoso lente crescentes, primum hyalinae, dein pallide flavo-brunnescentes, margine distincte myceliali; conidia numerosa, singula vel in capitulis in conidiophoris brevibus recumbentibus vel in lateribus hypharum formata; cellulae conidiophororum saepe disjungentes et ut conidia agerentes; gemmae e conidiis formatae; conidia conidiophoris et gemmis inclusis hyalina, unicellularia, 2.5μ–12.5μ longa, 2.2μ–5.0μ lata, plerumque elongata et paulo pyriformia; asci hyalini, paulo pyriformes, plerumque terminales et 2–6 caespitosi, interdum ad apicem vel nodos hypharum ascophorarum singuli, vel in capitulis compositis in ramis brevibus nati, 7.5μ–11.2μ longi, 6.2μ–10.0μ lati, bispori; ascosporae hyalinae, pileiformes costulatae, costula inculsa 6.2μ–9.8μ in diam., ad angulum rectum in costulam 1.8μ–3.2μ in diam., vulgo costulis e basi ad apicem extensis dispositae et ascum fere implentes.

The ambrosia in the insect tunnel consists of conidia and asci, at first hyaline, but, as larval activity increases, changing into a slimy yellowish coating on the tunnel wall. The adjoining wood-cell lumina become filled with hyphae and conidia, which turn to deep brown, causing an intense black stain of the wood adjacent to the tunnel. The reason for the darkening of hyphae and conidia in the wood was not determined, although inoculations showed that the color change occurred.

Ambrosia Fungus of Xyleborus affinis

An imperfect fungus, which apparently is the ambrosia of *Xyleborus affinis*, was observed in association with this beetle and was consistently isolated from tunnels and from adults. The fungus is apparently an undescribed species of *Cephalosporium* Corda.

Cephalosporium pallidum sp. nov.

On malt agar, colonies (fig. 3, *C*) are moderately slow growing, reaching 9 to 14 mm. in radius in 6 days at room temperature (75° to 85° F.). The margins are usually appressed and hyaline while the rest of the colony is covered with a thin layer of hyaline, fluffy aerial mycelium which often becomes appressed with age except for isolated tufts. Aerial mycelium may be entirely lacking. Occasionally a slight brownish tinge develops in parts of old cultures. Yellowish, yeasty mounds develop in aging cultures. In the yeasty mounds mycelium may be limited largely to pointed, short-celled hyphae (fig. 3, *A*) projecting but shortly from the yeasty mass of conidia and monilioid cells. Compact helicoid hyphal formations were commonly observed in the filamentous mycelium.

Conidia germinate on malt agar by forming monilioid chains of cells which finally give rise to hyphae (fig. 3, *A*). Spore heads are formed relatively soon after germination.

In culture typical fruiting consists of cephalosporic heads of conidia protruding but slightly above the agar (fig. 3, *A*) on erect or decumbent conidiophores. Conidiophores are usually unbranched and hyaline and terminate in 1 to 10 or more hyaline, unicellular conidia which are nearly spherical to slightly pear-shaped, 7.6μ–14.4μ long and 7.9μ–14.0μ wide, averaging 10.8μ × 10.4μ. When appreciable aerial mycelium occurs, conidiophores elongate and branch (fig. 3, *A*). Sometimes conidiophores are composed partly or totally of moniliform cells, particularly in the yeasty mounds. Occasionally buds were observed forming laterally on hyphae (fig. 3, *A*), and monilioid chains of spores of irregular sizes and shapes were observed in the agar or protruding above it (fig. 3, *A*).

Isolated from adults of *Xyleborus affinis* Eich. and from the wood adjacent to their tunnels. Observed in sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and southern sweetbay (*Magnolia virginiana* L.).

Coloniae in agaro maltoso modice lente crescentes; mycelium plerumque appressum et hyalinum, in culturis vetustis brunneotinctum; conidiophora plerumque non ramosa, hyalina, in conidiis 1–10 vel pluribus, hyalinis, unicellularibus, fere sphericis vel leniter pyriformibus, 7.6μ–14.4μ longis, 7.9μ–14.0μ

latis terminata, partim vel omnino e cellulis moniliformibus composita; gemmae interdum in lateribus hypharum formatae; catenulae monilioideae sporarum magnitudine formaque variabilium praesentes; conidia in natura singula vel 2–4 in capitulis, rare in catenulis brevibus nata.

The ambrosia (fig. 3, *B*) in the tunnels of *Xyleborus affinis* consists of conidia, mostly solitary, sometimes in heads of 2 to 4 spores and occasionally in short monilioid chains. In new tunnels single

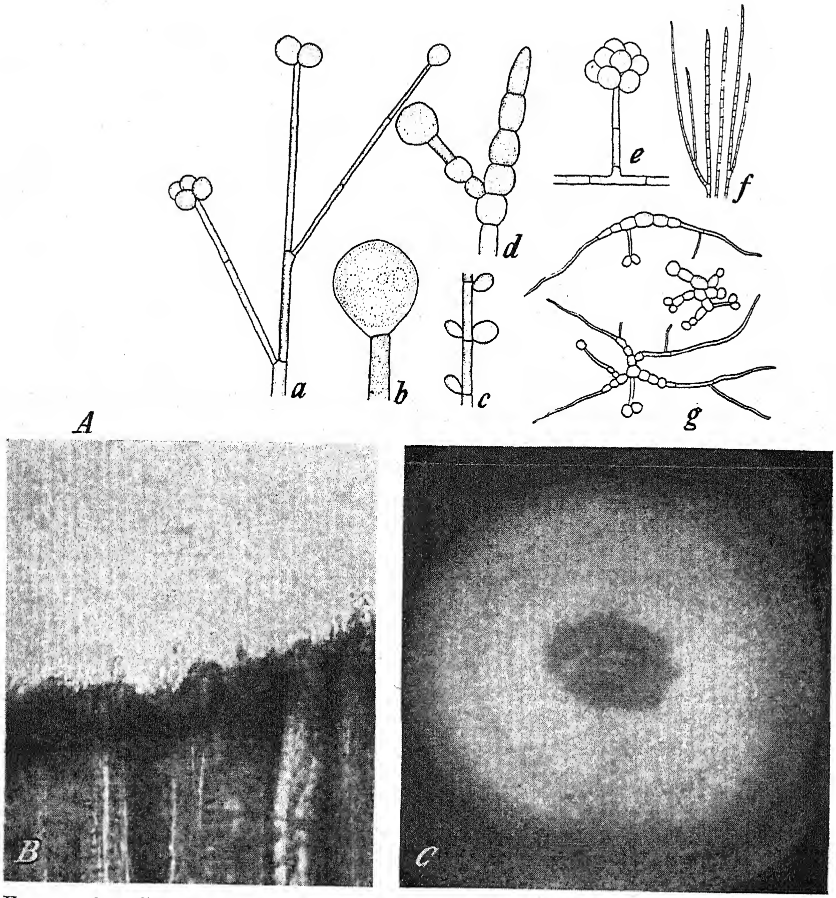

FIGURE 3.—*Cephalosporium pallidum*. *A*, Drawings from malt agar cultures: *a*, Branched conidiophore, × 320; *b*, single conidium, × 1,040; *c*, buds from hypha, × 320; *d*, monilioid cells, × 560; *e*, cephalosporic head, × 320; *f*, paraphyseslike hyphae, × 160; *g*, young colonies showing early production of conidia, × 120. *B*, Young ambrosia in insect tunnel, × 88. *C*, Culture 15 days old grown at about 80° F., × 0.8.

conidium-bearing conidiophores (12μ–38μ long) are easily seen, but later only a jumbled mass of cells is visible. The fungus mostly remains hyaline in the tunnel and adjacent wood cells, and does not produce stain around the tunnel except slightly in sweetgum and southern sweetbay.

The ambrosia fungus of *Xyleborus affinis* is apparently related to

that of *X. dispar*. The fungus associated with *X. dispar* was originally described by Hartig as *Monilia candida* (*1*). Neger (*4*) expressed the opinion that it may be an endomycete, although no asci or ascospores have been observed. Schneider-Orelli (*6*), in describing and illustrating the ambrosia fungus of *X. dispar*, shows it to be similar to that associated with *X. affinis* except that no mention is made of yellowish yeasty mounds or cephalosporic heads such as occur with the ambrosia fungus of *X. affinis*, although the author does illustrate a conidiophore bearing a single conidium similar to those in yeasty mounds of *Cephalosporium pallidum*. Furthermore, black stain occurs around the tunnels of *X. dispar* but is absent or not so pronounced around those of *X. affinis*.

Ambrosia Fungus of Xyleborus pecanis

The ambrosia fungus associated with the tunnels of *Xyleborus pecanis* is apparently an undescribed species of *Cephalosporium* Corda.

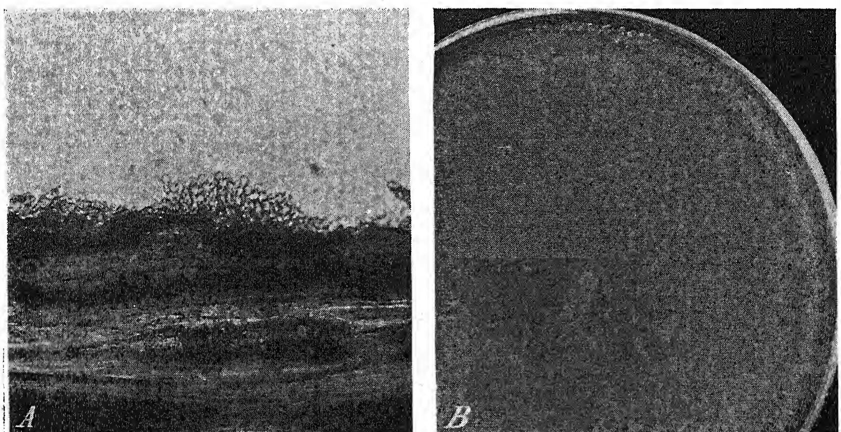

Fig. 4.—*Cephalosporium luteum:* *A*, Young ambrosia in insect tunnel, × 99; *B*, culture on malt agar, 15 days old, grown at about 80° F., × 0.8.

Cephalosporium luteum sp. nov.

On malt agar, colonies (fig. 4, *B*) are fast growing, reaching 60–70 mm. in radius in 6 days at 75° to 85° F. They are at first hyaline but soon become sulfur yellow to light brown. The agar is stained a deep brown. Aerial mycelium is at first fluffy but becomes appressed with age. No tendency for budding or yeasty growth was observed as with other ambrosia fungi encountered in this study. The margin of the colony is decidedly filamentous.

Fruiting on malt agar is sparse, and spores are often difficult to find. When a block of mycelium-bearing agar is removed from a culture and placed in a moist chamber, a few spores usually can be found along the cut edge on protruding conidiophores within 24 hours. Spores are formed on simple or branched conidiophores, mostly singly, sometimes in heads of two or three spores, are hyaline, nearly spherical, unicellular, and average 6.5μ in diameter. In nature spores are larger, 5.3μ–12.5μ wide and 6.1μ–15.0μ long, averaging $8.8\mu \times 10.1\mu$.

Observed in association with *Xyleborus pecanis* Hopkins in swamp tupelo (*Nyssa biflora* Walter) and southern sweetbay (*Magnolia virginiana* L.).

Coloniae in agaro maltoso rapide crescentes, primum hyalinae, dein e sulphuris brunneolae; mycelium aereum primum floccosum, vetustum appressum; fructificatio in culturis sparsa; conidiophora plerumque non ramosa et brevia; sporae

singulae vel 2–3 in capitulis, hyalinae, fere sphericae, unicellulares, in culturis 6.5μ in diam., in natura 5.3μ–12.5μ latae, 6.1μ–15.0μ longae, plerumque singillatim interdum 2–3 in capitulis natae.

The ambrosia in the beetle gallery is at first hyaline but later forms a yellowish to brownish lining to the brood chamber. It consists of mycelium and conidia (fig. 4, *A*), mostly borne singly on short, simple or branched conidiophores, or in heads of two to three spores. The conidia remain hyaline. The wood around the chamber is stained a light brown.

Young ambrosia is similar to that in the tunnels of *Xyleborus affinis*. Later, however, the ambrosia of *X. pecanis* turns a sulfur yellow and does not become yeasty, as does that of *X. affinis*. The conidia of the ambrosia fungus of *X. pecanis* are, in general, similar to some of the conidia of the ambrosia fungi of *X. affinis* and of *X. dispar*, but no monilioid chains, such as occur in the last two, were observed. The ambrosia fungus of *X. pecanis* is apparently the same as that of *X. xylographus* Say or is closely related to it, as illustrated and described by Hubbard (*2*).

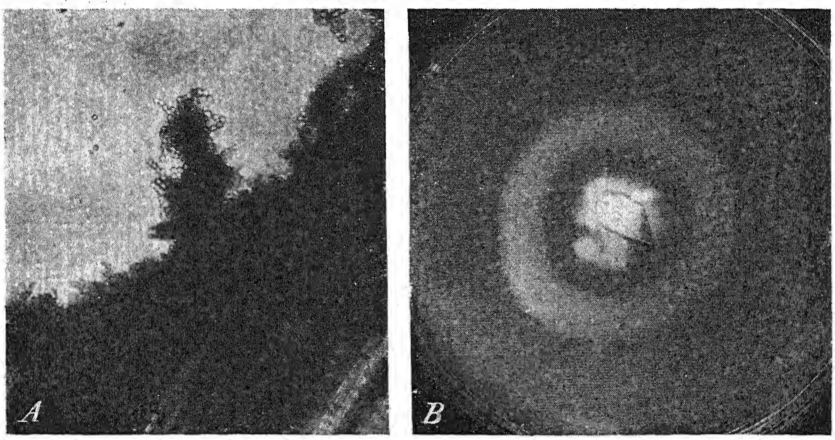

FIGURE 5.—*Monilia brunnea: A*, Ambrosia in insect tunnel, × 84; *B*, culture 15 days old grown on malt agar at about 80° F., × 0.7.

AMBROSIA FUNGUS OF PTEROCYCLON MALI AND P. FASCIATUM

The ambrosia fungus of *Pterocyclon mali* is apparently the same as the fungus associated with *P. fasciatum* and appears to be a new species of *Monilia* Pers.

Monilia brunnea sp. nov.

On malt agar, colonies are slow growing, reaching 8–10 mm. in radius in 6 days at 75° to 80° F. They are appressed, and at first are nearly hyaline but with age become dark brown (fig. 5, *B*). Original isolates were quite yeasty in general appearance and consisted largely of monilioid chains of rounded cells that budded in situ, similar to the growth described and illustrated by Leach et al. (*3*) for the ambrosia fungus of *Trypodendron betulae* Sw. and *T. retusum* (Lec.). After repeated subculturing, more filamentous mycelium was formed, although the monilioid type of growth persisted in parts of the culture. When filamentous mycelium was formed the surface of the culture became finely tomentose, the amount of aerial mycelium being limited. In old cultures there was a tendency

for small mounds to form, consisting mostly of monilioid cells. In one isolate short, pointed hyphae were formed, similar in shape to those described for the ambrosia of *Xyleborus affinis* but dark brown in color.

Monilioid cells are mostly hyaline, although in old cultures and old tunnels they may be distinctly brown, and are borne in simple or branched chains. Cells contain large granular particles. The terminal one to three cells are usually nearly spherical while the other cells are oblong or rectangular to spherical. Mature cells are 7.5μ–11.5μ long by 5.2μ–10.0μ wide, averaging 8.5μ in each direction. In some isolates there was a tendency in culture for conidia to collect in heads as well as in chains.

Spores germinate on malt agar by forming monilioid groups of cells that eventually give rise to hyphae.

Observed in association with *Pterocyclon mali* (Fitch) in sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), maple (*Acer* sp.), and oak (*Quercus* sp.), and with *P. fasciatum* (Say) in oak (*Quercus* sp.).

Culturae in agaro maltoso lente crescentes vetustae fuscescentes; mycelium plerumque appressum; sporae interdum gemmantes; hyphae in articulos oidiformes secendentes; sporae plerumque hyalinae, interdum in culturis vetustis et in viis insectorum brunneae, in catenulis simplicibus vel ramosis natae; sporae terminales 1–3 fere sphericae, alterae ex oblongis vel rectangularibus sphericae, 7.5μ–11.5μ longae, 5.2μ–10.0μ latae.

The ambrosia in the tunnels of the beetle consists of masses of monilioid cells (fig. 5, *A*) with little filamentous mycelium. Freshly formed ambrosia is hyaline, but in older tunnels it may turn dark brown. Ambrosia forms in both the main tunnel and the larval cradles. Wood cells adjacent to the tunnels become filled with hyphae and monilioid cells that soon turn brown, causing an intense black stain around the tunnel. Stain occurred in the wood of each species in which the beetles were found.

The ambrosia fungus of *Pterocyclon* apparently is closely related to that of *Trypodendron*. With both of these genera larvae are reared in separate cradles in contrast to the other genera encountered in this study, in which the larvae are free in the egg-laying tunnel. The ambrosia fungus associated with *Pterocyclon mali* and *P. fasciatum* apparently is a species of *Monilia* Pers. However, it is not identical with *Monilia candida* Hartig, associated with *Xyleborus dispar* (*6*), or the fungus described by Leach et al. (*3*) as the ambrosia of *Trypodendron betulae* and *T. retusum* and considered by those authors as a possible strain of *M. candida*.

SUMMARY

Four fungi commonly associated with southern species of ambrosia beetles are described as new species: *Endomyces bispora*, associated with *Platypus compositus; Cephalosporium pallidum*, with *Xyleborus affinis; C. luteum*, with *X. pecanis;* and *Monilia brunnea*, with *Pterocyclon mali* and *P. fasciatum*.

These fungi apparently are used as food by the ambrosia beetles with which they are associated and probably cause the restricted black or brown stain adjacent to the beetle tunnels.

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THE EFFECT OF SORGHUM KERNEL SMUTS ON THE DEVELOPMENT OF THE HOST[1]

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INTRODUCTION

Covered kernel smut, caused by *Sphacelotheca sorghi* (Lk.) Clint., is the most common and destructive disease of the sorghum crop in the United States. Although the loose kernel smut, caused by *Sphacelotheca cruenta* (Kühn) Potter, occurs occasionally in the United States, it is of less economic importance. Preventive measures for these smuts are well known, and no other cereal smuts may be more easily and economically controlled by means of specifics than these two. It is common knowledge that cereal plants affected with systemic smuts do not develop normally, but little is known of the cause of the abnormalities.

The purpose of the present study was to learn what morphologic changes occur in the organs of sorghum plants when attacked by these kernel smuts. The investigations at Manhattan, Kans., begun in 1929, were continued over a period of 7 crop years, during which great variations of temperature and precipitation occurred. Not all phases of the work, however, were carried on during the entire period. The present report covers the following phases of the work: (1) A study to determine the effect of *Sphacelotheca sorghi* and *S. cruenta* on the height of plant, diameter of stalk, and width of leaf of sorghum varieties; (2) a study of several varieties of sorghum infected with the two species of kernel smut to determine the parts of the host that are morphologically changed and to locate the regions responsible for a reduction in height; (3) a study of the number of nodes and tillers which occur in smutted and unsmutted plants of White Durra C. I. 81, Manchu Brown kaoliang C. I. 171, Evergeen Dwarf broomcorn C. I. 822, and Acme broomcorn C. I. 243; (4) a study of node differentiation in smut-infected plants; (5) observations on the vegetative proliferation of sorghum panicles following infection by *S. cruenta;* and (6) a study to obtain further information on the relation between size and shape of smut sori produced by the different physiologic races of *S. cruenta* on different varieties of sorghum.

REVIEW OF LITERATURE

In wheat affected with bunt, slight differences in height between diseased and normal plants, as well as other abnormalities, have been observed by various investigators (*1, 5, 8, 9, 16, 20, 23*),[2] but the cause of the difference in height between smutted and unsmutted plants has not been definitely determined. In recent years a physiologic race of *Tilletia tritici* has been described which produces a marked dwarfing

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[2] Italic numbers in parentheses refer to Literature Cited, p. 164.

of the wheat plant, but here again no report has been made on the cause of the reduction in height (*10, 24*). Viennot-Bourgin (*22*) found that in wheat bunt, a reduction in height of culms was due not so much to a shortening of the internodes as to a decrease in their number. Barrus (*2*) observed that wheat plants affected with bunt were shorter than healthy plants because of the general decrease in the length of the internodes, all internodes sharing equally in the decrease. He further observed that—

> In the case of wheat plants affected by loose smut where mature culms are shorter than healthy ones, the difference is due to the failure of the upper internode of affected plants to develop as much as healthy ones do.

It is known that the culms of smutted oat plants are frequently shorter than those of unsmutted plants of the same variety.

The literature bearing on the morphologic changes in the host that cause the reduction in height of smutted plants is not extensive, and the reports presented to date have been based upon observations rather than upon detailed studies. This is especially true of the kernel smuts of sorghum, *Sphacelotheca sorghi* and *S. cruenta* (*3, 4*).

Reed and Melchers (*19*) found that plants infected with *Sphacelotheca sorghi* headed as soon as normal plants and were essentially as tall as normal plants. On the other hand, plants attacked by *S. cruenta* were shorter than normal ones, headed earlier, and frequently produced more tillers. Infected plants also showed an enlargement of glumes and developed slenderer heads.

No detailed review of literature on the taxonomy or life histories of the two sorghum kernel smuts seems necessary; they are very similar (*19*). Primary infection occurs during the early growth of the sorghum seedlings, generally from germinating chlamydospores which adhere to the seed. After infection the mycelium invades the meristematic tissue of the seedling, keeping pace with the growing point. At flowering time and as soon as the sorghum heads emerge from the sheath, affected ovaries in the florets are easily recognized by the white smut galls (sori) and the absence of normal stamens.

METHODS AND MATERIALS

The seed of the sorghum varieties was obtained from self-pollinated heads and was considered pure for the variety. The smut was prepared and applied to the seed which was then planted and cultural crop practices were followed as described by Melchers (*12, 13*). Smutted and unsmutted plants grew from seed of the several varieties used in these investigations, and notes and measurements were taken when the plants had made their maximum growth. Fifteen smutted plants of each variety, or as many more as were available, were measured each year. No difficulty was encountered in measuring 15 or more unsmutted plants in the same row in which the smutted individuals grew. These served as check or control plants.

The height measurement was taken from the tip of the panicle to the base of the plant at the soil line. The diameter of the stalk was obtained just below the second leaf from the top of the plant by means of calipers. The diameter of the stalk in these studies includes the thickness of the stalk and leaf sheath. Leaf width was measured at its widest point and the second leaf from the top was always chosen for the measurement.

The varieties used belong to the main groups of sorghum; that is, forage, grain sorghums, and broomcorn. The experimental material consisted of such varieties and hybrids as Red Amber, Red Amber hybrids, and Kansas Orange among the sorgos; feterita, feterita hybrids, White Yolo, milos, Blackhull kafir, White Durra, and kafir × milo hybrid K. B. 2561 among the grain sorghums; Darso, Weskan, Schrock, Manchu Brown kaoliang, and Grohoma among the miscellaneous sorghums; and Acme and Evergreen Dwarf broomcorn.

The inoculum of *S. sorghi* consisted of a composite, in equal amounts, of three physiologic races (p. r. [3] 1, 2, and 3) of the organism known to attack the kafir, milo, and feterita groups, while the *S. cruenta* inoculum consisted of p. r. 1 and 2 used separately. These physiologic races have already been described (*12*, *15*).

EXPERIMENTAL RESULTS

EFFECT OF SMUT INFECTION ON HEIGHT OF PLANT, DIAMETER OF STALK, AND WIDTH OF LEAF

Although *Sphacelotheca sorghi* and *S. cruenta* are closely related, they react differently on the same variety of sorghum and vary in general appearance (*14*, *19*). Preliminary studies to determine the morphologic changes that occur in sorghum plants attacked by *S. cruenta* were begun in 1929. Two varieties of sorghum were of special interest that year because smutted plants were decidedly shorter than unsmutted plants. Measurements showed that the smutted plants of Manchu Brown kaoliang C. I. 171 had a mean decrease in plant height, stalk diameter, and leaf width of 54.2, 42.9, and 65.9 percent, respectively. Smutted plants of Acme broomcorn C. I. 243 had a mean decrease in plant height, stalk diameter, and leaf width of 45.0, 42.9, and 47.4 percent, respectively. These studies were then enlarged to include a representative group of 25 varieties of sorghum and both *S. cruenta* and *S. sorghi* were used. Four years' data obtained in 1931, 1932, 1935, and 1936 were averaged for each variety as shown in table 1.

There was some variability in height reduction among varieties of plants infected with *S. sorghi*, although the average reduction for the varieties taken as a group was only 1.9 percent. That *S. sorghi* does not greatly reduce the height of sorghum plants confirms the results obtained in other studies (*17*, *19*). In this connection Tyler (*21*) has shown that F_1 chlamydospore lines of *S. sorghi* may vary in respect to their ability to stunt sorghum during the parasitic phase. Acme broomcorn, kafir×feterita, Kansas Orange, Manchu Brown kaoliang, and Red Leaf feterita each showed a reduction in height of more than 10 percent, while the smutted plants of Weskan, Premo, Early White milo, kafir × milo 26–3–1–1, and Blackhull were taller than the unsmutted plants (table 1).

Both physiologic races of *S. cruenta* materially and consistently reduced the height of infected plants (table 1). Among the varieties tested this reduction ranged from a few to 38 percent for *S. cruenta* p. r. 1 and to 55 percent for *S. cruenta* p. r. 2, with the average for all the varieties for both physiologic races close to 18 percent (table 1).

[3] Physiologic race hereafter will be designated by the abbreviation p. r.

TABLE 1.—*The effect of sorghum kernel smut infection on the height, diameter of stalk, and width of leaf of sorghum plants, Manhattan, Kans., 1931, 1932, 1935, and 1936* [1]

Variety	Accession No.[2]	Average deviation from normal plant of plants attacked by—								
		Sphacelotheca sorghi p. r.[3] 1–3			*Sphacelotheca cruenta* p. r. 1			*Sphacelotheca cruenta* p. r. 2		
		Height of plant	Diameter of stalk	Width of leaf	Height of plant	Diameter of stalk	Width of leaf	Height of plant	Diameter of stalk	Width of leaf
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Acme broomcorn	C. I. 243	−14.7	−21.6	−12.5	−38.3	−58.5	−64.4	−37.3	−33.8	−33.9
Blackhull	K. B. 3047	+5.4	−17.5	−15.3	−8.6	−36.6	−17.6	−9.9	−27.3	−15.6
Darso	C. I. 615	−7.7	−21.9	−14.6	−8.2	−25.2	−15.9	−13.9	−19.7	−12.3
Dwarf Shantung kaoliang	C. I. 293	-----	-----	-----	−23.8	−25.0	−36.2	−18.7	−17.6	−21.0
Early White milo	C. I. 480	+7.2	−10.0	−13.3	−18.2	−43.0	−34.6	(4)	(4)	(4)
Feterita	S. P. I. 51989	+1.8	−20.4	−20.9	−13.0	−41.8	−38.9	−15.4	−38.5	−12.5
Feterita hybrid (feterita × kafir)	F. C. 8917	−5.3	−16.3	−21.1	−17.8	−36.7	−34.9	−27.1	−38.5	−33.3
Grohoma	C. I. 920	+2.4	−22.8	−12.2	−13.9	−30.2	−25.0	−2.2	−28.5	−25.8
Kafir × feterita	K. B. 2686	−13.9	−15.5	−14.8	−14.0	−38.3	−30.2	−11.9	−29.0	−30.0
Kafir × milo 26-3-1-1	K. B. 2561	+8.6	−22.8	−22.6	+.3	−18.5	−15.1	(4)	(4)	(4)
Kansas Orange	F. C. 9108	−12.4	−32.3	−16.6	−33.7	−45.7	−42.5	−24.2	−34.7	−32.4
Manchu Brown kaoliang	C. I. 171	−10.5	−22.8	−20.4	−28.0	−46.1	−42.2	−28.4	−34.9	−45.7
Pierce kaferita	K. B. 2547	−7.9	−18.6	−11.4	−13.3	−31.0	−31.7	−55.3	−28.6	−39.1
Premo	F. C. 8929	+12.6	−19.9	−16.5	−13.3	−35.9	−26.5	−15.6	−17.3	−20.4
Red Amber	F. C. 1534	+.5	−15.4	−8.4	−26.8	−30.3	−40.8	−19.6	−20.6	−14.1
Red Amber × feterita	K. B. 2570	+.9	−19.7	−24.9	(4)	(4)	(4)	−6.7	−19.5	−21.3
Do	K. B. 2562	−4.0	−21.4	−19.7	(4)	(4)	(4)	−14.2	−22.6	−14.8
Do	K. B. 2501	+.6	−20.2	−17.0	(4)	(4)	(4)	−16.4	−24.9	−22.4
Red Leaf feterita	K. B. 2543	−12.2	−11.8	−12.5	−18.9	−41.2	−36.8	−6.7	−25.2	−15.8
Schrock selection	C. I. 616	+3.2	−14.3	−9.9	−24.3	−38.8	−32.8	−15.2	−33.3	−13.5
Shallu	C. I. 85	−5.6	−20.0	−23.1	−25.8	−43.4	−30.2	−20.0	−26.8	−20.2
Weskan	K. B. 2522	+8.8	+2.3	−12.8	−8.3	−27.3	−33.1	−1.8	−26.8	−24.3
White Durra	C. I. 81	+1.4	−17.1	−12.4	−33.0	−51.4	−47.6	−22.8	−26.7	−25.0
White-seeded darso (selection).	K. B. 3002	−7.3	−18.5	−16.9	−10.2	−49.4	−22.8	−17.4	−24.4	−22.1
White Yolo	C. I. 699	+2.7	−14.8	−20.8	(4)	(4)	(4)	−18.2	−21.0	−23.8
Average of all varieties measured.		−1.9	−18.1	−16.3	−18.6	−37.8	−33.3	−18.2	−27.0	−23.4

[1] The figures given represent the averages of varieties grown 4 years (1931, 1932, 1935, and 1936), except Premo, which was grown 3 years.
[2] C. I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.
K. B. refers to Kansas Botany, Kansas State College, Manhattan.
S. P. I. refers to accession number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.
F. C. refers to accession number of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.
H. C. refers to cereal number of Fort Hays, Kans., Branch Experiment Station.
[3] p. r=Physiologic race.
[4] This sorghum seems to be immune, since no smut appeared in it for the years and the physiologic race of smut concerned.

Sphacelotheca sorghi brought about an average reduction in stalk diameter of 18 percent, while *S. cruenta* p. r. 1 and 2 caused reductions of 38 and 27 percent, respectively. Certain varieties showed an appreciable reduction in the diameter of stalk, while others were affected much less.

Sphacelotheca sorghi and *S. cruenta* exhibited a similar tendency with respect to reduction of leaf width, the former reducing it on an average 16 percent and *S. cruenta* p. r. 1 and 2 approximately 33 and 23 percent, respectively. In some instances the reduction exceeded the average leaf width of all the varieties.

From the data in table 1, one must conclude that the height of sorghums was not appreciably changed by *S. sorghi* infection, but that infection by *S. cruenta* did reduce it markedly (fig. 1, *A*). There was no great difference between the average percentage reduction in height of sorghum plants affected with *S. cruenta* p. r. 1 and 2; how-

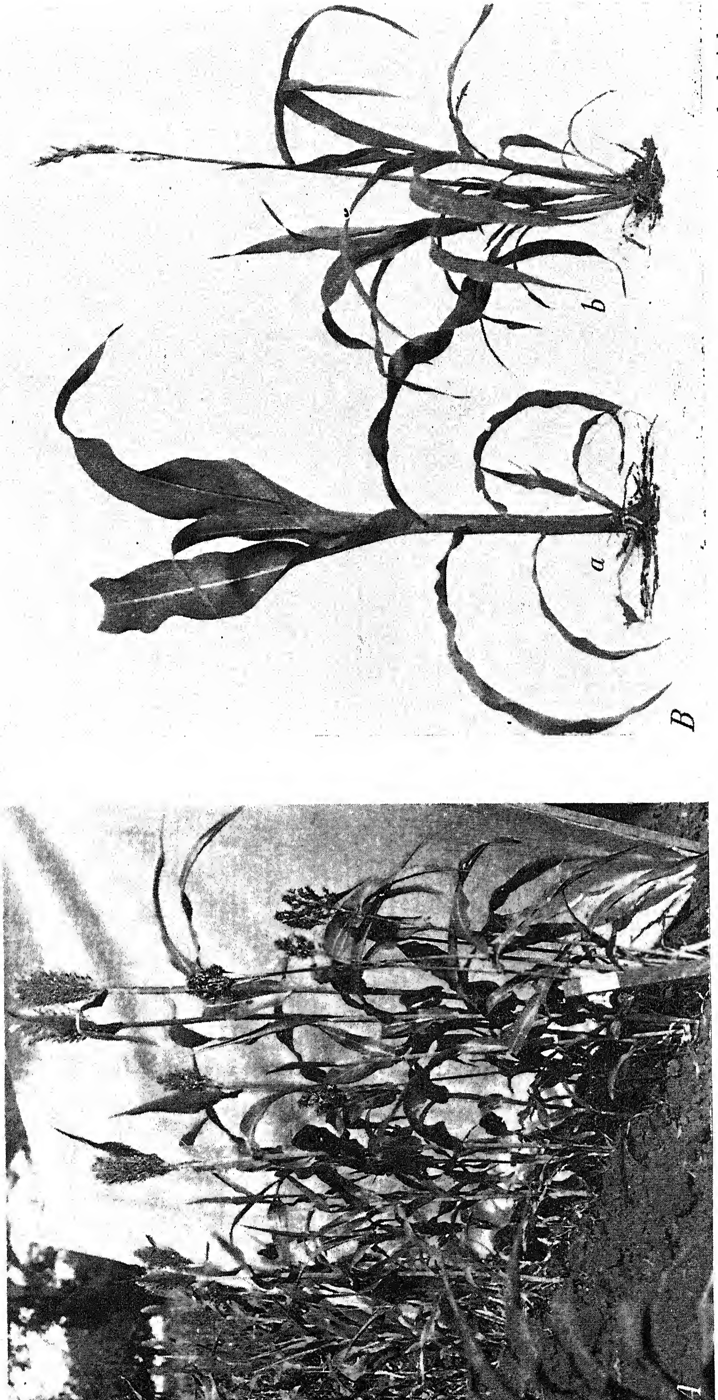

FIGURE 1.—*A*, Manchu Brown kaoliang, showing the effect of loose kernel smut of sorghum (*Sphacelotheca cruenta* p. r. 1) on the height of infected plants. The unsmutted plants had not made their complete growth when the photograph was taken; at a later date the contrast would have been greater. *B*, White Durra sorghum, showing the effect of smut infection on the growth of the host: *a*, Unsmutted plant; *b*, plant infected with *S. cruenta* p. r. 1. The plants were 1½ months old when the photograph was made.

ever, the average percentage reduction in diameter of stalk and width of leaves of plants affected with *S. cruenta* p. r. 1 was significantly greater than that for *S. cruenta* p. r. 2.

Reductions in height of plant, diameter of stalk, and width of leaf bring about a loss in total weight of plant, particularly in the case of attack by *S. cruenta.* It has been known for some time that the nutritive value of smutted sorghum plants is greatly reduced through the destruction of the grain but that the presence of sorghum smut itself is neither poisonous nor otherwise harmful to livestock when fed (*7*). The present studies show that considerably less tonnage of fodder is to be expected from plants attacked by *S. cruenta* and *S. sorghi,* particularly the former.

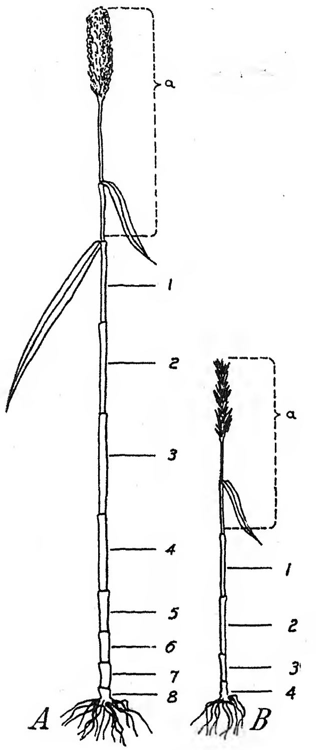

FIGURE 2.—Schematic sketch of a normal sorghum plant and of one infected with the loose kernel smut, *Sphacelotheca cruenta.* All but the topmost leaves have been removed, exposing the nodes. *A,* Normal plant with internodes numbered; *B,* plant attacked by *S. cruenta* showing reduced number of internodes; *a,* panicle and peduncle.

After these more general studies were completed, it seemed desirable to study in detail two varieties of sorghum to determine whether the difference in height between smutted and unsmutted plants was due to a reduction in the number of nodes per plant, to a difference in the length of the internodes, or to a combination of these factors. White Durra C. I. 81 and Manchu Brown kaoliang C. I. 171 were selected for this study.

Reed and Faris (*18*) observed that Valley kaoliang affected with *S. cruenta* had shorter stems with fewer nodes than normal plants or than those infected by *S. sorghi.* Measurements of internode lengths and counts on the number of nodes of smutted and unsmutted plants were made in 1936 at Manhattan, Kans., on White Durra C. I. 81 and Manchu Brown kaoliang C. I. 171. A study was also made of the effect of the two species of smut on the length of the peduncle [4] and panicle of the sorghum plant and on the length of the four internodes below the peduncle in order to locate specifically the regions responsible for difference in height. Since most of the plants attacked by *S. cruenta* did not produce over four or five nodes above the soil line, it was thought best not to consider comparative measurements below the fourth node.

Figure 2 is a schematic sketch of a normal sorghum plant and of one attacked by the loose kernel smut. The various parts of the plant used in the measurement studies are illustrated. The comparative average lengths of the panicle and peduncle combined (fig. 2, *A and B, a*), as well as the length of the first to fourth internodes inclusive, of normal and smutted plants are presented in table 2.

[4] The peduncle is the part of the stem joining the panicle with the first node below the panicle.

TABLE 2.—*Results showing effect of sorghum kernel smut infection on length of peduncle and panicle combined, and on each of the 4 internodes below the peduncle of the sorghum plant, Manhattan, Kans., 1936*

WHITE DURRA C. I. 81

Sorghum kernel smut	Length of peduncle and panicle		Length of internode below peduncle: First		Second		Third		Fourth		Combined length [1]	
	Normal	Smutted	Normal	Smutted	Normal	Smutted	Normal	Smutted	Normal	Smutted	Normal	Smutted
	Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
S. sorghi p. r. 1-3	50.8	48.4	13.1	12.3	12.2	10.7	11.5	10.8	12.2	11.6	99.8	93.8
S. cruenta p. r. 1	56.7	59.3	15.5	21.8	12.1	11.1	11.4	7.8	12.7	5.4	108.4	105.4
S. cruenta p. r. 2	54.5	53.4	14.2	15.2	12.8	12.1	12.8	10.4	11.0	9.9	105.3	101.0
MANCHU BROWN KAOLIANG C. I. 171												
S. sorghi p. r. 1-3	53.0	55.1	13.7	17.2	15.9	15.5	16.7	15.7	15.9	14.0	115.2	117.5
S. cruenta p. r. 1	48.5	47.8	12.1	15.8	13.3	11.7	13.8	8.7	15.9	6.9	103.6	90.9
S. cruenta p. r. 2	52.9	43.8	15.9	13.6	16.0	11.9	15.8	9.7	14.6	7.8	115.2	86.8

[1] Panicle, peduncle, and first 4 internodes below.

There was no marked difference in the combined length of peduncle and panicle of the normal and smutted plants in the two varieties White Durra and Manchu Brown kaoliang for either species of smut, with perhaps the exception of *S. cruenta* p. r. 2 on Manchu Brown kaoliang. The greatest difference occurred in internode length of plants attacked by *S. cruenta* p. r. 2, although those infected with p. r. 1 showed the same trend (table 2). If only *S. cruenta* is considered, there is an appreciable difference between smutted and normal plants in internode length below the peduncle, but the variation in length in comparable internodes is not always consistent. It is, however, consistent for the third and fourth internodes of both varieties, and a marked reduction in the length of these internodes in the smutted plants is noted (table 2). Other varieties of sorghum, such as Acme broomcorn, were just as severely stunted by *S. cruenta* as the two varieties considered here.

As stated previously, smutted plants developed fewer nodes. In a comparison of infected and normal plants, corresponding internodes were not comparable as to position. For example, internode 4 of a plant smutted with *S. cruenta* was essentially at the ground line while internode 4 was about the middle of a normal plant (fig. 2).

Significant differences may be noted for *S. cruenta* p. r. 1 and 2 if the combined lengths from the tip of the panicle, to and including the fourth internode, of smutted and normal plants of Manchu Brown kaoliang are considered (table 2). Some differences are also noted in White Durra but these are less striking. These figures do not represent the total height of plants but rather that part from the tip of the panicle down to and including the fourth internode. The reduced height in plants infected with *S. cruenta* apparently was due in part to the shorter internodes, but the entire reduction in height cannot thus be explained. Smutted plants in the field were frequently about one-half the height of normal plants of the same variety (fig. 1, *A*).

The effect of smut on the total number of nodes visible above the ground was analyzed and the results are given in table 3. The data are expressed in terms of the smutfree [5] plants. Plants of the same varieties attacked by *S. sorghi* had approximately the same number of nodes as unsmutted plants. The same varieties, however, when infected with the two physiologic races of *S. cruenta* had fewer nodes (table 3). The average decrease was 3.5 and 1.5 nodes in plants attacked by *S. cruenta* p. r. 1 and 2, respectively. The reduced height of smutted plants apparently was due more to the reduced number of nodes than to the length of the internodes, according to the 1936 data (table 3).

TABLE 3.—*Results showing effect of sorghum kernel smut infection on the number of nodes in the sorghum plant, Manhattan, Kans., 1936*

Treatment	Average number of nodes per plant [1]		Average decrease in number of nodes
	White Durra C. I. 81	Manchu Brown kaoliang C. I. 171	
S. sorghi p. r. 1-3:			
Smutfree	7.5	8.6	
Smutted	8.5	7.7	0
S. cruenta p. r. 1:			
Smutfree	7.7	7.5	
Smutted	3.7	4.5	3.5
S. cruenta p. r. 2:			
Smutfree	6.9	8.0	
Smutted	6.2	5.8	1.4

[1] Visible nodes above ground.

In 1937 the studies were enlarged to include four varieties of sorghum. Data on the height of plant, diameter of stem, leaf width, and the number of nodes in smutted, smutfree, and check [6] plants of the varieties Evergreen Dwarf broomcorn C. I. 822, Acme broomcorn C. I. 243, White Durra C. I. 81, and Manchu Brown kaoliang C. I. 171 are given in table 4. The comparisons were based on smutfree plants, although either smutfree or check plants could have been used with no appreciable difference in the final results.

An analysis of the data in table 4 shows that in smutted plants the total length of panicle and peduncle and the four internodes below was generally less than that of the smutfree plants. The difference usually was less in plants attacked by *S. sorghi* than in those attacked by *S. cruenta*. The reductions in length of internode could not in themselves, however, be responsible for the great reduction in total height of smutted plants. Similar conclusions were reached from the studies in 1931, 1932, 1935, and 1936. The measurements included only the length of the plants from the tips of the panicles down through the fourth internodes.

[5] "Smutfree" means that plants grew from treated seed and were free from internal smut infection.

[6] Check plants grew from untreated seed and produced normal panicles. Since some of them may have carried infection in the stalk even though the heads were not smutted, a smutfree control was used in 1937 as a safeguard in making comparative measurements.

TABLE 4.—*Results showing effect of sorghum kernel smut infection on length of panicle and peduncle combined, length of 4 internodes below the peduncle, total height of plant, diameter of stem, width of leaf, and number of nodes per plant, Manhattan, Kans., 1937*

Variety	Treatment	Length of panicle and peduncle	Length of internode below peduncle				Combined length of panicle, peduncle, and internodes 1 to 4	Height of entire plant	Reduction in height of smutted plant	Diameter of stem	Width of leaf	Average nodes per plant [1]	Average decrease in nodes per plant [2]
			First	Second	Third	Fourth							
Evergreen Dwarf broomcorn C. I. 822.	*S. sorghi* p. r. 1–3:	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Pct.*	*Cm.*	*Cm.*	*No.*	*No.*
	Smutted	62.3	7.6	6.6	8.0	9.1	93.6	139.4	4.5	1.1	6.4	8.6	0.8
	Smutfree	69.8	7.3	6.8	7.6	8.8	100.3	145.9	-----	1.4	7.8	9.4	-----
	Check	66.6	6.0	6.7	7.7	8.3	95.3	143.8	-----	1.4	7.5	9.4	-----
	S. cruenta p. r. 1:												
	Smutted	47.3	8.0	10.0	9.8	9.5	84.6	84.2	44.0	.6	3.5	4.7	4.9
	Smutfree	69.5	8.0	6.3	7.7	8.3	99.8	150.3	-----	1.5	7.4	9.6	-----
	Check	73.8	7.0	8.3	8.9	9.8	107.8	153.0	-----	1.6	7.9	9.2	-----
	S. cruenta p. r. 2:												
	Smutted	57.2	9.5	9.0	9.0	9.2	93.9	104.1	32.7	1.0	3.8	5.8	3.7
	Smutfree	73.8	7.0	7.2	8.4	8.9	105.3	154.6	-----	1.6	7.8	9.5	-----
	Check	70.9	8.0	8.6	9.5	9.2	106.2	149.8	-----	1.6	7.8	10.2	-----
Acme broomcorn C. I. 243.	*S. sorghi* p. r. 1–3:												
	Smutted	71.4	9.0	10.1	9.5	9.4	109.4	151.9	6.6	1.2	6.1	9.1	.9
	Smutfree	82.6	9.0	9.6	9.2	9.2	119.6	162.7	-----	1.8	7.4	10.0	-----
	S. cruenta p. r. 1:												
	Smutted	70.0	11.0	8.0	8.0	9.0	106.0	136.0	14.7	1.3	5.5	8.0	1.7
	Smutfree	77.5	7.0	9.2	9.1	9.1	111.9	159.4	-----	1.7	7.3	9.7	-----
	S. cruenta p. r. 2:												
	Smutted	58.5	10.0	7.0	7.0	9.5	92.0	106.5	30.6	1.1	4.7	6.2	3.5
	Smutfree	73.3	9.0	8.6	9.6	9.4	109.9	153.4	-----	1.7	7.3	9.7	-----
	Check	72.0	8.0	7.9	8.8	8.9	105.6	155.0	-----	1.6	7.6	10.0	-----
White Durra C. I. 81.	*S. sorghi* p. r. 1–3:												
	Smutted	60.4	15.2	16.1	16.2	14.8	122.7	141.1	11.5	1.1	6.8	6.2	.1
	Smutfree	66.2	21.2	16.9	17.1	16.1	137.5	159.4	-----	1.3	7.8	6.3	-----
	Check	68.4	22.0	19.3	16.7	16.0	142.4	167.2	-----	1.3	8.2	6.7	-----
	S. cruenta p. r. 1:												
	Smutted	63.6	22.0	21.5	14.5	12.0	133.6	115.4	26.1	.9	5.5	3.8	3.0
	Smutfree	65.2	20.0	17.3	18.0	17.0	137.5	156.2	-----	1.4	8.1	6.8	-----
	Check	67.5	19.8	19.4	17.6	16.2	140.5	162.0	-----	1.5	8.4	6.6	-----
	S. cruenta p. r. 2:												
	Smutted	57.1	18.5	17.4	15.5	12.9	121.4	115.8	24.5	1.0	5.6	5.2	1.9
	Smutfree	67.3	21.4	18.8	15.9	15.4	138.8	153.3	-----	1.5	8.5	7.1	-----
	Check	66.8	35.0	10.0	19.4	18.0	149.2	141.6	-----	1.3	12.8	6.8	-----
Manchu Brown kaoliang C. I. 171.	*S. sorghi* p. r. 1–3:												
	Smutted	62.4	22.0	17.6	17.8	16.8	136.6	150.3	8.9	1.2	6.6	6.5	.3
	Smutfree	59.8	18.2	19.9	18.8	19.3	136.0	165.0	-----	1.3	7.6	6.8	-----
	Check	58.8	19.0	19.3	19.2	17.4	133.7	164.9	-----	1.2	7.6	6.6	-----
	S. cruenta p. r. 1:												
	Smutted	47.8	10.8	9.0	12.2	11.6	91.4	93.0	35.3	.7	4.5	4.8	1.3
	Smutfree	55.8	17.5	17.2	16.6	18.6	125.7	143.8	-----	1.1	7.2	6.1	-----
	Check	54.5	13.5	13.5	14.4	14.2	110.1	142.6	-----	1.2	7.5	6.5	-----
	S. cruenta p. r. 2:												
	Smutted	64.0	19.0	15.5	16.0	14.5	129.0	147.0	17.4	1.1	6.9	6.0	1.0
	Smutfree	63.8	17.6	19.8	19.0	18.9	139.1	177.9	-----	1.3	8.5	7.0	-----
	Check	60.8	16.5	16.8	16.6	17.5	128.2	172.0	-----	1.4	8.2	6.8	-----

[1] Above ground or visible nodes.
[2] The difference in number of nodes is based on "smutfree" and smutted plants.

Sphacelotheca sorghi reduced the height of the plant slightly, while *S. cruenta* p. r. 1 and 2 reduced it materially, as already shown in these studies. The percentage reduction for smutted plants of the four varieties infected by the two species of smut showed a range for

S. sorghi of 4.5 to 11.5 percent; for *S. cruenta* p. r. 1, 14.7 to 44 percent; and for p. r. 2, 17.4 to 32.7 percent. These reductions in height are illustrated by figure 1, *A*, showing plants of Manchu Brown kaoliang infected by *S. cruenta* p. r. 1.

The average number of nodes per plant for each variety and the average decrease in the number of nodes in smutted plants as compared with smutfree plants are given in the last two columns of table 4. The average decrease in number of nodes for the four varieties was 0.5, 2.7, and 2.5 in plants affected with *S. sorghi*, *S. cruenta* p. r. 1, and *S. cruenta* p. r. 2, respectively (table 4). Plants affected with *S. sorghi* had essentially the same number of nodes above ground as the smutfree plants. This explains why they were approximately the same height. Plants infected with *S. cruenta* always had fewer nodes, and in many instances only half as many nodes, as smutfree plants.

While the reduced height of smutted plants may be brought about by a combination of shortened internodes and fewer nodes, the latter factor is the more important. The two species of smut differ markedly in the extent to which they effect reductions in different varieties, but the general tendency is for *S. cruenta* definitely to dwarf the host.

The reduction in the stalk diameter of smutted plants of the four varieties was significant in most cases and in accord with previous results.

The effect on leaf width was most pronounced in the case of *S. cruenta* infection, although it was also noticeable in the case of *S. sorghi*.

EFFECT OF SMUT INFECTION ON GROWTH CYCLE

A striking characteristic of sorghum infected with *S. cruenta* is the tendency for smutted plants to head several days to 2 weeks earlier than unsmutted plants (*3, 19*). One of the field characteristics of the development of the loose kernel smut of sorghum is for the host to speed up its growth cycle. In figure 1, *B*, which shows plants of White Durra at 1.5 months of age, the smutted plant (*b*) has headed, while the noninfected plant (*a*) is still about 2 weeks from heading. This difference in time of heading between smutted and normal plants was very much less noticeable in plants attacked by *S. sorghi;* generally such plants do not head more than a day or two before the normal plants.

EFFECT OF SMUT INFECTION ON NODE DIFFERENTIATION

A possible explanation for the differences observed in node development in smut-infected and normal plants may be derived from histologic studies. In the present investigations the embryos of Western Blackhull kafir, Darso, Scarborough broomcorn, Pygmy milo, and feterita were removed from dormant seed and from seed which had germinated for 24 hours. It was found that about five nodes besides the coleoptilar and scutellar nodes had differentiated in the embryo of the normal sorghum seed, a condition similar to that occurring in the embryo of corn and other grasses. Supporting evidence by Evans and Grover (*6*) is found in their morphologic studies of eight species of grasses other than sorghum.

As the seed germinates and grows, additional nodes are successively differentiated at regular intervals in the apical meristem of the plant.

After the rudiments of the panicle are differentiated, no additional nodes are formed. This differentiation occurs early in the ontogeny of the plant. The number of nodes in the embryo of inoculated or uninoculated sorghum seed of the same variety or strain would naturally be the same. Some of the young plants are infected with the smut as the inoculated seed germinates. The smut invades and follows the meristematic tissues of the plant as the latter grows to maturity, affecting its metabolism in such a way that fewer nodes are formed prior to the differentiation of the panicle. The plant heads earlier than the unsmutted plant and is dwarfed, a condition which is due primarily to the reduction in the number of internodes.

In this connection a supplementary experiment was conducted in the field and in the greenhouse in 1941 with 10 varieties of sorghum. The seed was not smutted, since the authors wished to study the normal growth of the plant. By varying the environmental conditions an average increase of 50 percent was obtained in the number of nodes in the greenhouse plants as compared with the same varieties in the field. It is believed, therefore, that the number of nodes which a sorghum plant eventually develops depends partly on the inherent nature of the variety or strain and partly on environmental factors which affect the general metabolism of the plant.

Since plants affected with *S. cruenta* are much more dwarfed than those affected with *S. sorghi*, the invasion of the meristematic tissues of the seedling by *S. cruenta* probably had a greater effect on the metabolism of the varieties and strains tested than did *S. sorghi*, and consequently a greater influence on node development.

It is not known whether a chemical depressant is given off by the specific smut fungus that causes the host to respond in this manner, or whether the mere association of the mycelium of the smut fungus in the meristematic tissues of the plant is partly responsible for the differentiation of fewer nodes. The former explanation seems the more plausible. Different varieties and strains of sorghum respond differently to the same smut. The same variety or strain may respond differently to the two races of *S. cruenta* and *S. sorghi* (table 4).

EXCESSIVE TILLERING

A difference in tillering, very pronounced in a few varieties, was observed between smutted and unsmutted plants. All stalks or culms are considered as tillers in this discussion. A summary of the data on 25 varieties of sorghum shows that plants attacked by *S. cruenta* p. r. 1 had an average of 1.4 more tillers per plant than normal plants; those attacked by *S. cruenta* p. r. 2 had 0.6 more tillers, and those attacked by *S. sorghi* had 0.5 more tillers, than normal plants. Tillers produced by plants attacked by *S. cruenta* were short, slender, and sometimes decidedly "Sudan grasslike." A striking example of this is shown in figure 3, *A*, *b*, typical for Shallu C. I. 85. A representative picture of what occurs in many varieties, in this instance Acme broomcorn, is shown in figure 3, *B*, *a*.

If the varieties White Durra, Manchu Brown kaoliang, Shallu, and Acme broomcorn are considered from the point of view of tillers produced, it will be found that those attacked by *S. cruenta* p. r. 1 produced excessive tillering, while those attacked by *S. cruenta* p. r. 2 and *S. sorghi* showed only a moderate increase in tillering. Two

FIGURE 3.—On some varieties of sorghum the effect of loose kernel smut (*Sphacelotheca cruenta* p. r. 1) is to produce excessive tillering. *A*, *a*, A normal plant of Shallu; *b*, a smut-infected plant of Shallu. The "Sudan grasslike" but more prostrate growth is a striking feature of this variety (photographed Oct. 20, 1936). Such prolific tillering is not typical of all varieties. *B*, *a*, Acme broomcorn, showing the increased tillering characteristic of many sorghum varieties attacked by *S. cruenta*; *b*, normal plant of Acme variety (photographed Aug. 8, 1930).

years' data were obtained, but since the general tendency was the same for each year, the results for 1936 only are given. The data in table 5 show the comparative differences in amount of tillering for these varieties on August 18. Had the number of tillers been counted in October when the photograph (fig. 3, *A*) was taken, the number in both normal and smutted plants would have been greater than those given in table 5. It is apparent that a smutted plant has a tendency to produce more as well as shorter shoots. This tendency varies in varieties and may be influenced both by seasonal conditions and the age of the plant.

TABLE 5.—*Results showing the effect of smut infection in sorghums on tiller production, Manhattan, Kans., 1936* [1]

Smut species and sorghum variety	Number of tillers per plant		Increase in number of tillers
	Normal	Smutted	
S. sorghi p. r. 1–3:			
White Durra	2.1	1.9	−0.2
Manchu Brown kaoliang	2.0	2.6	.6
Shallu	2.3	3.8	1.5
Acme broomcorn	1.7	3.1	1.4
S. cruenta p. r. 1:			
White Durra	1.6	3.9	2.3
Manchu Brown kaoliang	1.4	3.3	1.9
Shallu	2.6	4.9	2.3
Acme broomcorn	1.3	6.1	4.8
S. cruenta p. r. 2:			
White Durra	1.5	3.0	1.5
Manchu Brown kaoliang	1.5	1.9	.4
Shallu	2.3	3.5	1.2
Acme broomcorn	1.8	3.7	1.9

[1] Data taken August 18.

VEGETATIVE PROLIFERATION OF SMUTTED PANICLES

During the course of these investigations, it was observed that pronounced proliferation of smutted panicles occurred in certain varieties of sorghum attacked by *Sphacelotheca cruenta* (fig. 4, *A, b*). Reed (*17*) and Reed and Melchers (*19*) mentioned this as a characteristic of this species of smut. In the experimental plots in Kansas increased proliferation has not been observed in unsmutted panicles of the same varieties growing in the same row, nor has it been noted in plants attacked by *S. sorghi.* A similar, but usually more intensified proliferation has been observed in Kansas and elsewhere on sorghum plants attacked by *S. reilianum* (Kühn) McAlp., which causes head smut (fig. 4, *A, a*). Apparently this abnormal condition is the expression of a stimulus brought about by conditions other than smut infection, since Karper and Stephens (*11*) describe a similar, heritable abnormality on unsmutted Blackhull kafir panicles in Texas. In 1940 several plants of Wheatland sorghum collected in Stevens County, Kans., had a type of proliferation not due to smut infection. The writers believe this may be similar to the one described by Karper and Stephens (*11*). A normal panicle of Wheatland, a greatly proliferated panicle, and a deformed spikelet are illustrated in figure 4, *B*.

The proliferation caused by the loose kernel smut infection is more pronounced in some varieties than others. In the group of 25 varieties examined over a period of several years, those in which proliferation

FIGURE 4.—*A*, Two types of proliferation brought about by smut infection in sorghum: *a*, Head smut (*Sphacelotheca reilianum*); *b*, loose kernel smut (*S. cruenta*). *B*, *a*, Normal head of Wheatland sorghum; *b*, deformed spikelet; *c*, greatly proliferated panicle. This proliferation was not due to smut infection; possibly it has a genetic explanation.

was observed to be very marked were Shallu, Acme broomcorn, Kansas Orange sorgo, and Coon darso. Essentially, the abnormality is a type of fasciation with small shoots developing from tissue which generally gives rise to the floral organs of the spikelet. A smutted panicle with numerous abnormal spikelets becomes very noticeable because of the "brushy" appearance of the panicle (fig. 4, *A*, *b*). The pronounced dark-green color of the proliferated part contrasts vividly with the normal green of the remainder of the plant. It is similar to the dark-green color that develops in heads of wheat plants attacked by *Tilletia levis*.

The extreme case of panicle proliferation shown in figure 5, *A*, is a plant of kafir×feterita infected with *S. cruenta* p. r. 2, which was selected by C. O. Johnston, who made a further study of its development. Some of the smutted spikelets vegetated rapidly after rains in late August. No seed developed in the head. A few spikelets produced anthers, as shown in figure 5, *A*, *a*. The spikelet shown in figure 5, *A*, *b* was removed, placed in water, and allowed to stand in the laboratory where it produced roots, as shown in figure 5, *B*. Later it was placed in soil in the greenhouse, but it lived only a few weeks. On dissection, it was found to contain two or three aborted spikelets, which seemed to indicate that the structure was proliferated flowering parts.

SIZE OF SMUT SORI

Considerable variation has been observed in the size of the smut sori on different varieties of sorghum. This feature was not studied in detail, but it is apparent that the sori on certain varieties are particularly long and curved. The differences in size and color of the smut sori and peridia of different physiologic races of *S. sorghi* have already been described (*15*). Recently observations on the size of sori and color of peridia have been made by Tyler (*21*) in connection with genetic studies in *S. sorghi*.

When kafir × feterita K. B. 2686 was infected with *S. cruenta* p. r. 1 and 2, there was a marked difference in the appearance of the sori of the two races. Several years' data showed that the average length of the sori of p. r. 1 was about 1 cm., while that of p. r. 2 was about 1.5 cm. (fig. 6, *A*). The differences were not seasonal. The variation in length of the sori on other varieties of sorghum inoculated with the two physiologic races of *S. cruenta* was less noticeable, although there was a tendency for those produced by p. r. 1 to be slightly longer. Only 2 years' study has been made on this phase of the work, and further observations with other varieties would be desirable.

Figure 6, *B*, shows certain differences between physiologic races 1 and 2 of *S. cruenta* on White Durra. These differences were observed for several seasons on this variety. Figure 6, *B*, *e*, is a normal head of White Durra and *f* is smutted, showing glume proliferation and the absence of awns. Smutted panicles of White Durra in which the sori involve the rachilla (fig. 6, *B*, *a* to *d*, and *g* to *j*) have been observed during certain seasons on a few varieties affected with *S. cruenta* physiologic race 2, but not on the same varieties affected with *S. cruenta* physiologic race 1 under the same environmental conditions.

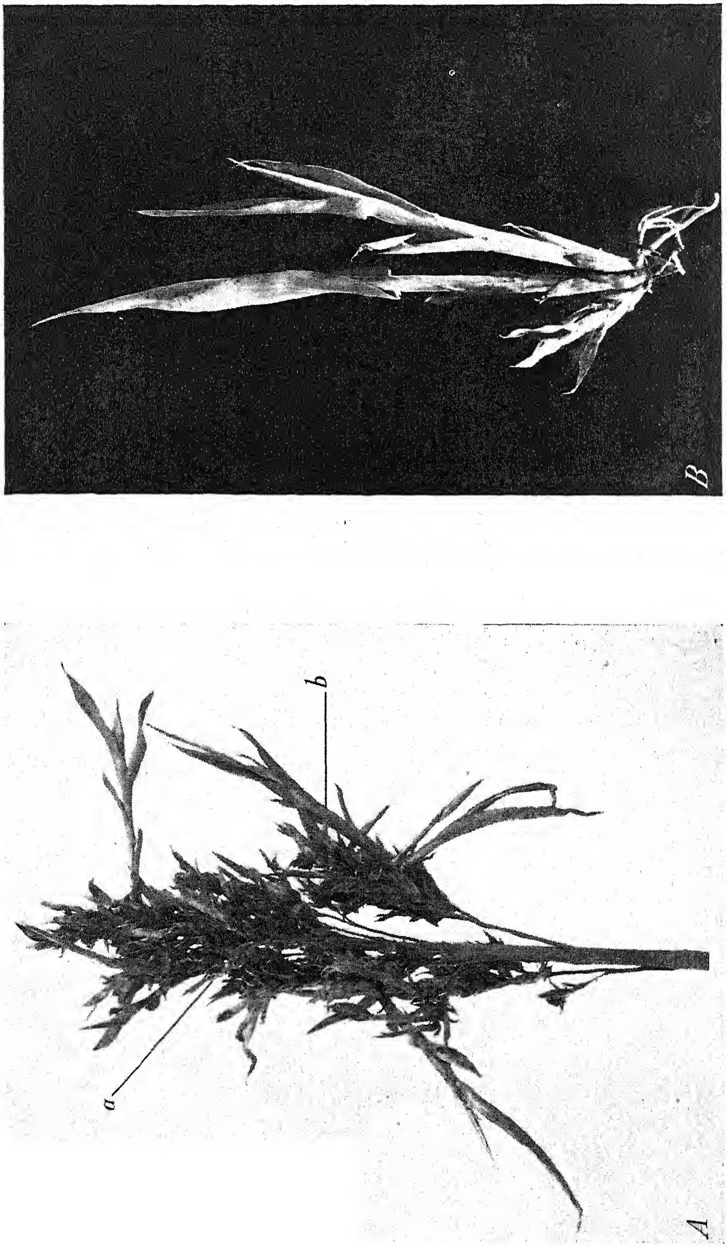

FIGURE 5.—Loose kernel smut of sorghum causes pronounced proliferation of sorghum panicles in some instances. *A*, *a*. A normal spikelet showing anthers; *b*, a proliferated spikelet (plant) which was removed and placed in water. *B*, The same spikelet as that shown at *A*, *b*; it developed roots when placed in water but did not survive when planted in soil. (Photograph by C. O. Johnston.)

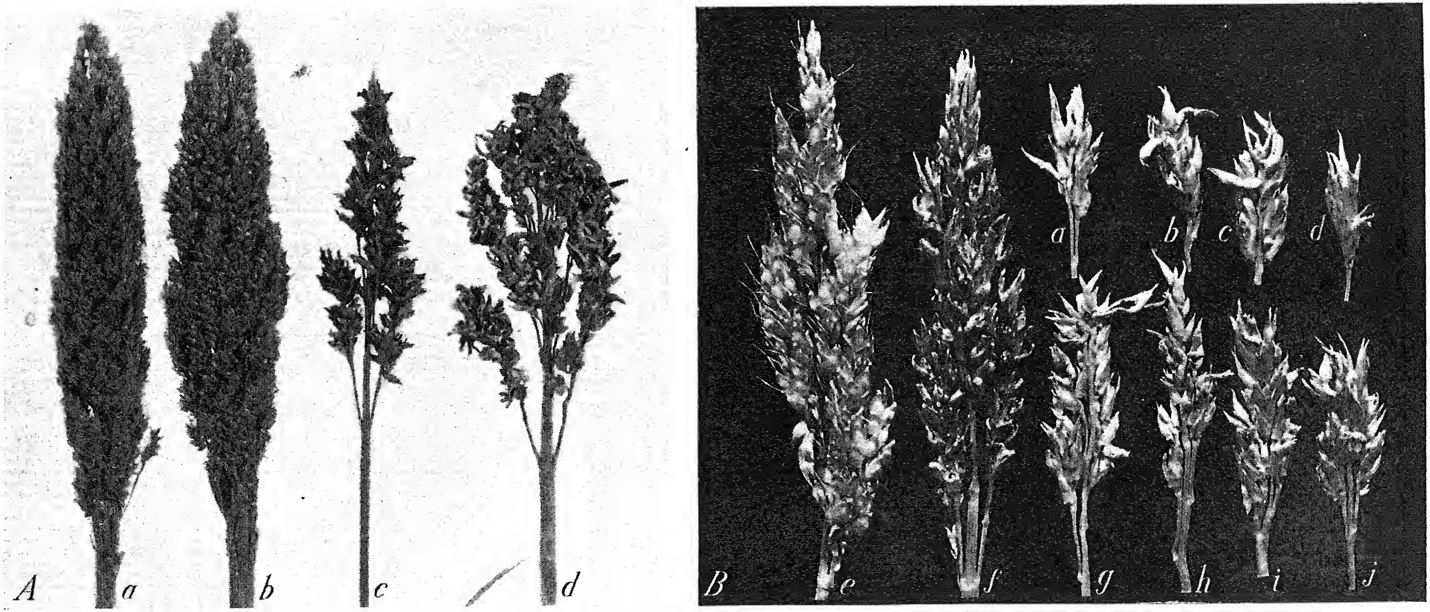

FIGURE 6. *A.* Normal and smutted panicles of a hybrid, kafir×feterita K. B. 2686, collected on the same day, showing differences in the size and appearance of the smut sori of two physiologic races of *Sphacelotheca cruenta*. *a.* Unsmutted panicle. Note stamens, proof that smut is not present. *c.* Panicle taken from the same row as *a*, attacked by *S. cruenta* p. r. 1. b. Unsmutted panicle for comparison with smutted panicle *d*, both selected from the same row. The smut in *d* is *S. cruenta* p. r. 2. Compare the large, curved, or horn-shaped sori with the fragile, moderate-sized, straight sori of p. r. 1 shown in *c*.

B. Morphologic differences between the sori of *S. cruenta* p. r. 1 and 2 on White Durra (X 2); *a*, *b*, *c*, and *d*, and *g*, *h*, *i*, and *j*, Branches of panicles affected with *S. cruenta* p. r. 2, showing the tendency of the sori to involve the rachilla. This condition was common in 1937 with this race of smut on White Durra. *e*, A normal panicle of White Durra. *f*, A panicle of White Durra affected with *S. cruenta* p. r. 1 in which the sori are confined to the ovaries; note the proliferation of the glumes.

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In some varieties infected with physiologic race 1, the fungus limits its sporulating area to the ovaries of the flower, as shown in figure 6, *B*, *f*.

LACK OF AWN DEVELOPMENT

The group of sorghums known as milos have short awns or barbs on the glumes, a characteristic which distinguishes them from other

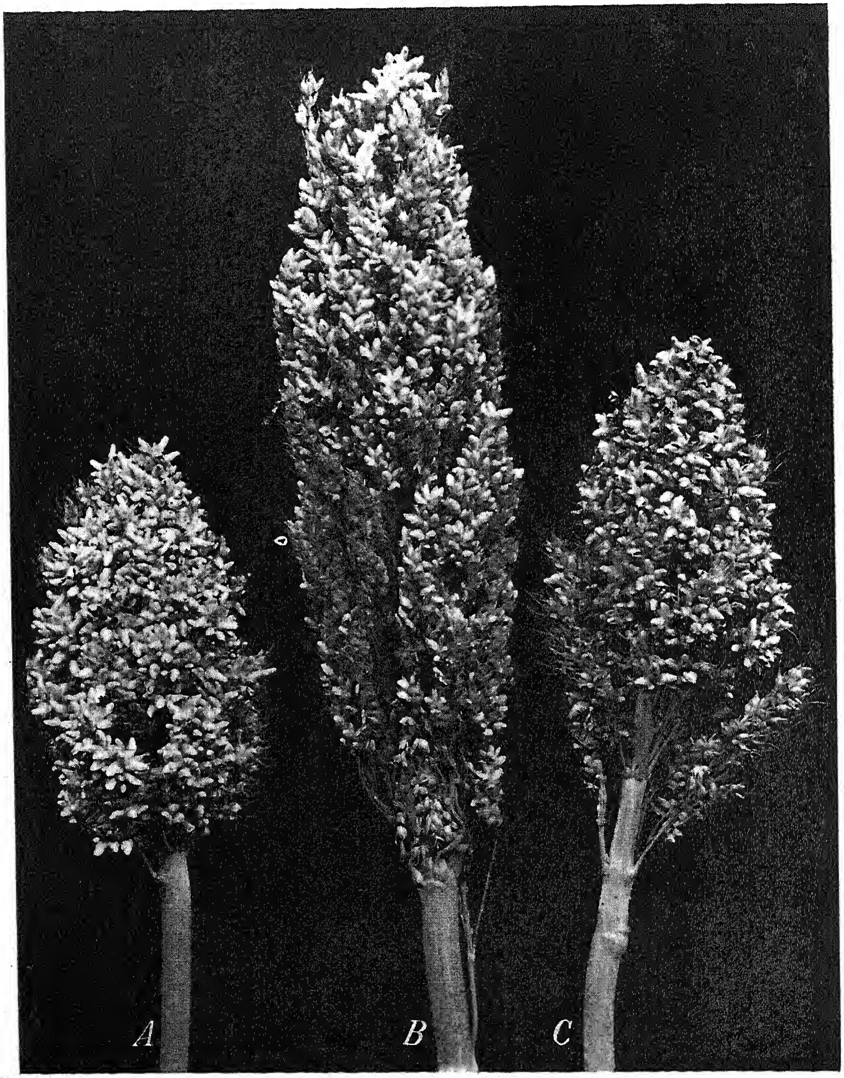

FIGURE 7.—Partly smutted panicles of Sooner milo, (*A*), Darso (*B*), and Cream milo (*C*). The smut is *Sphacelotheca sorghi* physiologic races 2. Note the lack of development of the awns on the glumes of the smutted spikelets. This is characteristic of the milo group.

sorghums. The milos are generally considered resistant to *S. sorghi* and *S. cruenta*, although recent studies have proved that certain physiologic races attack them (*14*, *15*). When a milo head or part of

a panicle is infected with the kernel smut organism, there is a partial or entire lack of awn development on the spikelets attacked, as is shown in figure 7. The fungus in this instance was *S. sorghi* physiologic race 2; the effect of *S. cruenta* physiologic race 1 on White Durra is shown in figure 6, *B, f*. In the latter case there was a complete suppression of awn development since all the spikelets were affected by the smut.

SUMMARY AND CONCLUSIONS

A study was made of 25 varieties, selections, and hybrids of sorghum to determine the effect of the kernel smuts *Sphacelotheca sorghi* and *S. cruenta* on the normal development of the host. Data on the reduction in height of plant, diameter of stalk, and width of leaf showed that sorghum varieties react differently to these two smuts. The average reduction in height from the normal of the varieties tested with *S. sorghi* was 2 percent; with *S. cruenta* physiologic races 1 and 2, 19 and 18 percent, respectively. The average reduction in diameter of stalks infected with *S. sorghi* was 18 percent, whereas in stalks infected with *S. cruenta* physiologic races 1 and 2 the average was 38 and 27 percent, respectively. The average reduction in leaf width of smutted plants as compared with the normal for all the varieties infected with *S. sorghi* was 16 percent; while with *S. cruenta* physiologic races 1 and 2, it was 33 and 23 percent, respectively. In general, therefore, the reductions in height, diameter of stalk, and width of leaf were greater in plants infected with *S. cruenta* than in those infected with *S. sorghi*. Furthermore, *S. sorghi* infection did not consistently reduce the height of the plant, although it materially reduced the diameter of the stalk and the width of the leaves.

These reductions in plant parts by both of the kernel smuts are of economic importance since they result in a reduction in the tonnage of grain and forage that may be expected from badly smutted crops.

A study was made to find what causes the reduction in height of smutted plants. The reduced height of plants infected by *S. cruenta* was found to be due partly to shortened internodes, but primarily to a reduced number of internodes. Plants attacked by *S. cruenta* had fewer nodes than those attacked by *S. sorghi*. In several instances the varieties attacked by the former had only half as many nodes as smut-free plants.

A histologic explanation of the causal factors concerned in node reduction of smut-infected sorghum plants is believed by the writers to be as follows. The smut fungus invades and follows the apical meristematic tissue of the sorghum plant. This invasion affects the metabolism of the growing plant in such a way that it forms fewer nodes prior to the differentiation of the panicle than does a normal plant. Consequently the infected plant heads earlier than the noninfected plant and is dwarfed primarily because of the reduction in the number of internodes. Whether it is chiefly a chemical stimulus initiated by the specific smut fungus that causes the host to respond in this manner, or whether some additional action of the mycelium in the meristematic tissue is partly responsible is not known. The authors believe the first explanation to be the more plausible. In respect to the extent of node reduction, varieties and strains of sorghum

differ. Also, the same variety or strain may differ in its response to attack by the different races of *S. cruenta* and *S. sorghi*.

The growth cycle of plants attacked by *S. cruenta* is speeded up; the plants head from a few days to about 2 weeks earlier than unsmutted plants. In general, plants attacked by *S. sorghi* head at approximately the same time as smut-free plants.

In 25 varieties of sorghum plants attacked by *S. cruenta* physiologic race 1 had an average of 1.4 more tillers than normal plants; the plants attacked by *S. cruenta* physiologic race 2 had 0.6 more tillers; and those attacked by *S. sorghi* had 0.5 more tillers. The results indicate a tendency for smutted plants to tiller more abundantly than smut-free plants, and in certain varieties this characteristic is very pronounced.

The proliferation of glumes of some varieties of sorghum infected with *S. cruenta* was very striking in the field because of the brushlike appearance and abnormally dark-green color of the glumes. Plants attacked by *S. sorghi* showed neither of these characteristics.

In the experiments conducted, the size and shape of sori were found to vary according to the species and race of smut and the variety of sorghum attacked. However, too few varieties were studied for definite conclusions to be formed and additional data would be desirable. It is apparent that kafir × feterita K. B. 2686 when infected with *S. cruenta* physiologic race 2 has longer and more curved sori than when affected with *S. cruenta* physiologic race 1 or *S. sorghi*.

A singular effect of sorghum kernel smut on the florets of milo and milo hybrids is the lack of development of awns which is a characteristic of normal florets in this group of sorghums.

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EFFECT OF BORON DEFICIENCY ON THE HISTOLOGY OF GARDEN BEET AND CABBAGE [1]

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INTRODUCTION

When garden beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* var. *capitata* L.) are grown in nutrient solution or in soil deficient in boron, marked external malformation, together with internal discoloration and necrosis, occurrs in several organs of the plants. The appearance of macroscopic symptoms is quite sudden if boron deficiency is acute. Signs of the disease are similar in seedlings of both species and in the mature plants of garden beet, but they differ markedly in the mature plants of cabbage. Since beet and cabbage differ widely in anatomy, they were particularly suitable subjects for a comparative study of the nature and extent of histological changes leading to and accompanying macroscopic symptoms of boron deficiency.

Investigators undertook to study the histological changes induced in plants by boron deficiency soon after establishment of the fact that boron is essential to normal plant growth. Interest in the subject was further stimulated by the discoveries of Mes (*20*) [3] and Brandenburg (*4*) that a number of unexplained plant diseases were due, primarily, to boron deficiency. The effects of boron deficiency have been investigated by others in sugar beet (*Beta vulgaris* L.) (*21*), broadbean (*Vicia faba* L.) (*5, 32*), potato (*Solanum tuberosum* L.) (*24*), tobacco (*Nicotiana tabacum* L.) (*22, 26*), tomato (*Lycopersicon esculentum* Mill.) (*15, 23*), pea (*Pisum sativum* L.) (*28*), rutabaga (*Brassica campestris* var. *napobrassica* DC.) (*6, 17*), carrot (*Daucus carota* L.) (*33*), sugarcane (*Saccharum officinarum* L.) (*19*), corn (*Zea mays* L.) (*7*), apple (*Malus pumila* Mill.) (*18*), grapefruit (*Citrus grandis* Osbeck) (*12*), and orange (*Citrus sinensis* Osbeck) (*12*). These reports vary from mere mention of affected tissues to detailed histological studies.

These studies showed that the most extensive pathological changes caused by boron deficiency occur in the meristematic regions and in the pith. Frequently the first evidence of degeneration appeared at or near the apical growing point. Later, abnormalities were common in vascular tissues throughout the plant and in the mesophyll of expanding leaf laminae. Often differentiation was retarded or suppressed. Younger differentiated elements were misshapen, and hypertrophy and hyperplasia, accompanied or followed by necrosis, were common in the cambial zone. Some workers found inter- and intracellular deposits in the necrotic areas. The histological changes found

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[2] The writers wish to acknowledge the helpful advice of Prof. Emma L. Fisk, Department of Botany, University of Wisconsin, during the course of this investigation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 181.

in boron-deficient sugar-beet seedlings by Rowe (*21*) were of particular interest to the writers because of the anatomical similarity between the sugar beet and the garden beet. A comparison between these and the abnormalities found in garden beet will be brought out later in this paper.

MATERIALS AND METHODS

Most of the plants used in these studies were grown in sand-nutrient cultures. The remainder came from a field of Poygan silty clay loam near Winneconne, Wis., or from one of Miami silt loam at Madison, Wis.

The clay pots in which the plants were grown were given two coats of varnish to prevent them from absorbing salts. A one-half inch layer of small stones was placed in the bottom of each pot to insure good drainage. A thin layer of glass wool was placed over the stones, and the pots were then filled with washed white quartz sand.

In preliminary work in sand-nutrient culture, Shive's Best three-salt solution [4] (*25*), to which small amounts of ferrous sulfate ($FeSO_4.7H_2O$), zinc sulfate ($ZnSO_4.5H_2O$), manganous chloride ($MnCl_2.4H_2O$), and copper sulfate ($CuSO_4.5H_2O$) were added, was diluted 10 times and used as the standard. The solution was modified [5] in later experiments to obtain more vigorous growth, but no marked differences in the histology or morphology of the plants grown in the original and the modified solution were observed. These two solutions will be referred to as minus-boron solutions. When it was desired to obtain normal growth, boric acid (H_3BO_3) was added to the solution at the rate of 0.75 p. p. m. of boron. A modification of the continuous-flow system described by Allison and Shive (*1*), adjusted to deliver approximately 1 liter per day to each 10-inch clay pot, was used to supply the solution. The salts and water used in these studies were found by microchemical test [6] to be free from boron.

Plants started from seed in minus-boron sand-nutrient cultures seldom developed more than two or three true leaves. Therefore, when plants at later stages of development were desired, it was necessary to grow them for some time in the complete solution before transferring them to the minus-boron solution. Small beet plants grown in normal nutrient, including those with five or six leaves, were removed and their roots washed free of sand. Some were then transplanted to minus-boron solution and others back to complete solution to give plants in both types of culture the same start. A better method for larger plants was the shifting of entire pots from the complete to the minus-boron solution. When this shift was made a certain amount of boron was carried over in the sand and the appearance of boron-deficiency symptoms was delayed 2 to 4 weeks. Cabbage plants were handled similarly, except that seedlings up to the eighth-leaf stage were transplanted. In all cases in which plants were transferred from one solution to the other, similar plants were left under the original conditions as controls.

Garden beets of the varieties Detroit Dark Red (Ferry strain), Good For All, and Woodruff Improved Short Top were used in the

[4] $MgSO_4.7H_2O$, 0.0150 M; KH_2PO_4, 0.018 M; and $Ca(NO_3)_2.4H_2O$, 0.0052 M.

[5] $MgSO_4.7H_2O$, 0.0004 M; KH_2PO_4, 0.0016 M; and $Ca(NO_3)_2.4H_2O$, 0.0016 M; this solution was used without dilution. The same microelements as used in addition to Shive's Best solution were incorporated.

[6] The writers are indebted to Dr. Kermit C. Berger, Department of Soils, University of Wisconsin, for making these tests.

field and greenhouse studies. The cabbage varieties used were Jersey Queen, Resistant Detroit, All Head Select, Marion Market, Wisconsin All Seasons, and Wisconsin Ballhead.

Care was exercised to collect material as nearly comparable as could be obtained from normal and boron-deficient plants, taking into account the relatively slower growth of those in the deficient nutrient solution.

After preliminary trials with several fixatives, a solution of formalin, ethyl alcohol, and acetic acid, mixed in the ratio of 5–90–5, was selected. All material was prepared for infiltration by the ethyl-butyl alcohol method and then embedded in tissue-mat paraffin with a melting point at 52° to 54° C. Relatively thick sections were required in many cases since the pathological changes induced by boron deficiency caused the tissue to crumble and to tear easily. Most of the material was stained with safranine and fast green. For study of certain structures of the phloem in beets, safranine and orange G, Delafield's haematoxylin and safranine, and a modification of Mayer's haemalum and orange G were used.

EXPERIMENTAL RESULTS

PATHOLOGICAL HISTOLOGY OF BORON-DEFICIENT GARDEN BEET

The macroscopic symptoms of the boron-deficiency disease, known as internal black spot, have already been described (*29*, *30*). The normal anatomy of the sugar beet has been described fully by Artschwager (*2*, *3*), Esau (*8*, *9*, *10*, *11*) and Hayward (*14*). In general the facts apply also to the garden beet. The histology of boron-deficient plants will be discussed in relation to the various organs affected.

Root and Hypocotyl

The effect of a deficiency of boron on root and hypocotyl is considered first with regard to plants in which little or no tertiary thickening had occurred. The most noticeable abnormality at this stage was found in certain cells of the phloem resembling companion cells. Many of these were entirely or partly filled with a dense substance which stained heavily with safranine, orange G, or haematoxylin (pl. 1, *B*, *C*). The lumen contents were densest near the cell wall. Near the center of the cell they were granular or made up of a mosaic of dark- and light-stained areas. Similar degenerated phloem cells were found in the roots of plants showing a marginal necrosis of the lower leaves which resulted from an excessive supply of boron (pl. 1, *D*). This suggests that the response is not specific for boron deficiency.

In content these cells were similar to those described by Rowe (*21*) in the phloem of sugar-beet seedlings receiving an inadequate boron supply. Some of them abutted the sieve tubes and appeared to be companion cells rather than the abnormal sieve tubes found by Rowe (*21*) in boron-deficient sugar beets. Others were not associated with the sieve tubes and their identity remains uncertain. They may be phloem-parenchyma or undifferentiated phloem cells, but in any case they do not appear to be sieve tubes. In normal plants the dense cytoplasm in the companion cells stained heavily, but except near the nucleus the lumen was not completely filled at any one level, and the contents were not as dense as the material in the cells of boron-

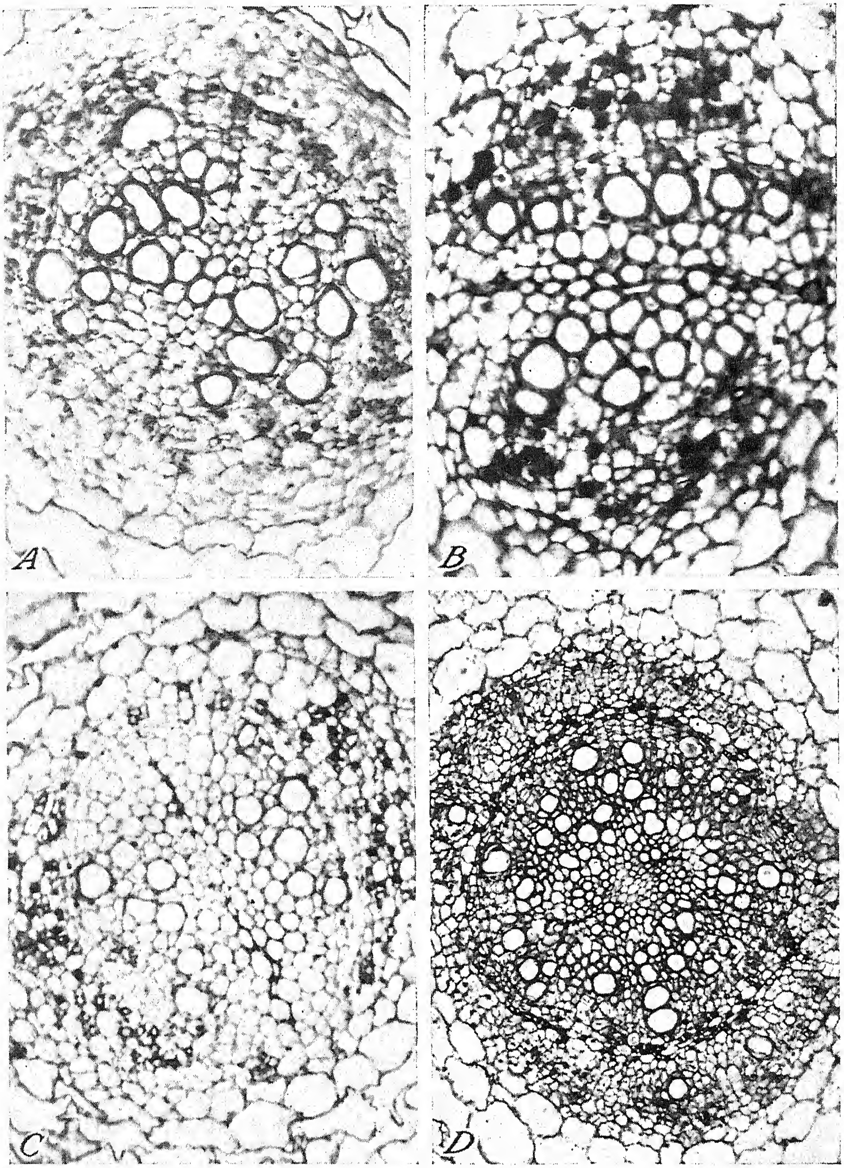
deficient plants filled with darkly stained material. As a result, relatively few heavily stained cells were seen in cross sections of normal plants (pl. 1, *A*) as compared with the number in abnormal plants (pl. 1, *B*, *C*). Usually the other tissues of the root and hypocotyl of boron-deficient plants were normal at this stage, but occasionally large thin-walled cells replaced the normal tabular cambial cells between the secondary xylem and phloem. In a few specimens a number of the cells which would normally function in initiating the first tertiary ring were hypertrophied and had proliferated while adjacent endodermal cells were crushed.

In slightly older plants thick-walled xylem elements had differentiated in the first two tertiary rings and the phloem could just be distinguished in the third ring. Pathological changes were more extensive in these than in younger plants. In the secondary phloem the degenerated cells were quite numerous. In both primary and secondary xylem intercellular brown deposits appeared, particularly near the thick-walled xylem elements, some of which were filled with darkly stained material. Cells adjoining the deposits were frequently distorted in size and shape. In the first tertiary ring, discolored or degenerated phloem cells were fairly numerous. The disorganization in the xylem of this ring was more pronounced than in the secondary xylem, especially in proximity to differentiated vessels. Here groups of parenchyma cells had discolored walls and contained discolored protoplasts. Many such cells had collapsed. Near the periphery of such areas there was less discoloration of both walls and protoplasts, while enlargement and proliferation of bordering parenchyma cells tended to wall off the necrotic region from the normal tissue. Although most of the pathological areas included only two or three thick-walled xylem elements and adjacent parenchyma cells, a few involved all the xylem and cambium of a bundle or of two adjacent bundles. When the cambium was included, the normal cells were replaced to some extent by spherical to cubical thin-walled cells.

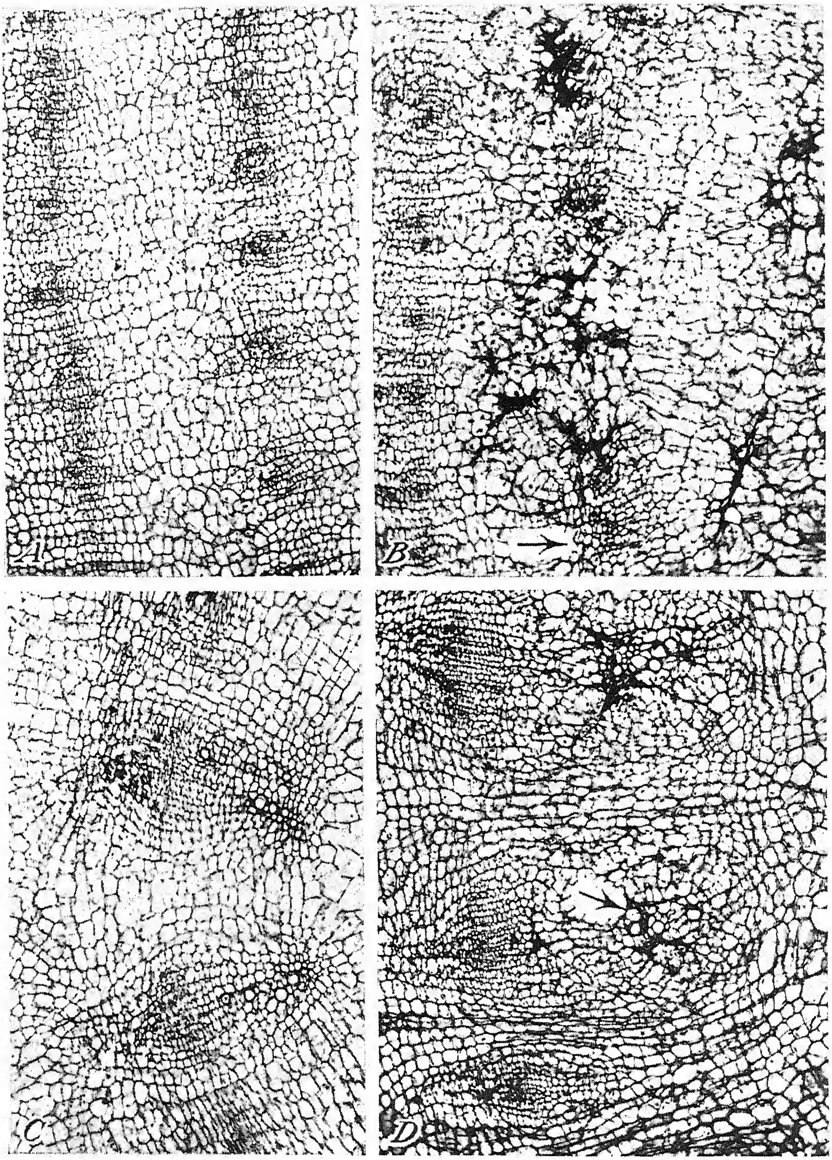
The second and third tertiary rings in some plants at this stage were normal; in others there was slight cell proliferation or enlargement and a few discolored and crushed cells in some of the bundle initials. These occurred near the few differentiated thick-walled elements in the second ring and in an occasional phlœm group in the third ring. It is probable that the degree and location of the pathological changes in these rings were determined by the relative acuteness of boron deficiency in the plants examined. No abnormalities were found in the fourth ring, in which only a few phloem cells had differentiated.

Plants similar to those just described were continued on boron-deficient nutrient and examined 1 month later to determine the effect of a longer period of boron starvation. By this time there was considerable interzonal parenchyma in the first four rings. A few thick-walled vessels were present in some bundles of the third tertiary ring of the "taproot" [7], but only the phloem groups had differentiated in the fourth and fifth rings. In the well-differentiated bundles of the first and second rings the pathological changes and tissues involved were the same as those described for the first ring of younger plants except that a greater proportion of the xylem of affected bundles was usually involved (pl. 2, *D*). At this stage in the third

[7] "Taproot" as used here includes root and hypocotyl.



Transections of garden beet seedlings. *A,* Lower hypocotyl of normal plant. X 155. *B,* Lower hypocotyl of boron-deficient plant; note deeply stained phloem cells. X 310. *C,* Lower hypotocyl of boron-deficient plant. Many of the abnormal phloem cells are only partly plugged. Compare with *A* and *B.* X 207. *D,* Hypocotyl of plant receiving an excess of boron; note numerous cells filled with darkly stained materials in the secondary phloem and the early differentiation of xylem elements in the tertiary ring. X 105.



Transections from "taproot" of garden beets in which the fifth tertiary ring had begun to differentiate. *A*, Fourth and fifth rings in normal beet. X 51. *B*, Fourth and fifth rings in boron-deficient beet; note that while a portion of the fourth ring is entirely disorganized, some vascular groups are discernible in ring (indicated by arrow). X 42. *C*, Normal beet, second tertiary ring. X 42. *D*, Boron-deficient beet, second tertiary ring; note that abnormalities in xylem and adjacent parenchyma are associated with thick-walled xylem elements (indicated by arrow). X 44.

and fourth rings there were some abnormal areas as in the younger plants, but there were also larger areas of disorganized tissues involving all or part of several bundles and adjacent interzonal parenchyma in one or both rings (pl. 2, *B*). Scattered throughout such areas were groups of crushed parenchymatous cells enclosing brown deposits among them as well as an occasional misshapen vessel segment, sieve tube, and companion cell. Near the margin of these areas the brown deposits were for the most part intercellular, some cells appearing normal except for a slight discoloration of one or two walls. Interspersed among the patches of necrotic cells were groups of small rapidly dividing cells and enlarged, thin-walled ones. Those adjacent to the necrotic tissues sometimes appeared to be so oriented as to wall off that region. However, the larger areas of proliferated cells which were arranged in radial rows appeared to be part of the degenerate area rather than the result of a growth response to the necrosis. Many of the larger cells had their long axis in a horizontal plane. The few isolated normal phloem groups in disorganized areas probably had differentiated before boron deficiency became acute. The areas of disorganized tissues in the third and fourth rings were macroscopically visible as typical internal black spot lesions, while those in the two inner rings were not, indicating that a considerable amount of the brown deposit and many necrotic cells were necessary before there was any macroscopic evidence of the pathological changes in the boron-deficient plants. No abnormalities were found in the fifth tertiary ring of plants at this age.

In still older plants which had three or more tertiary rings in which xylem elements had differentiated, pathological changes were confined to two or three rings much as was found to be the situation in sugar beets by Rowe (*21*). The fact that the inner rings were usually normal suggested that they had differentiated before the available boron was used up. In the ring in which only a few thick-walled xylem elements had developed and in the next one or two outer rings there were disorganized and necrotic areas, similar to, but more extensive than, those in comparable rings of younger plants. Frequently the ratio of necrosis to hypertrophy and hyperplasia was greater in older plants. The youngest outer rings in which a few bundle initials were present were normal.

Field-grown beets showing internal black spot symptoms contained abnormalities similar to those just described (pl. 3). The apparent confinement of symptoms to interzonal parenchyma in larger field beets can be attributed in part at least to suppression of vascular differentiation in the affected area, to an increased amount of interzonal parenchyma, and to a higher ratio of necrosis to proliferation in the affected parenchyma. In some field specimens, disorganized and necrotic areas appeared in the more mature inner rings with or without degeneration in the younger rings. It is probable that there was a deficiency in boron during the period of rapid development in the affected areas and that the supply of boron became adequate again before much differentiation occurred in succeeding normal rings. In roots of some plants abnormalities were confined to a single small area in one layer of interzonal parenchyma. The abnormalities found were the same as those in larger spots except that the area was completely separated from normal tissue by a zone of actively dividing, thin-walled cells.

In larger beets degenerated areas in the interzonal parenchyma frequently assumed a definite pattern (pl. 3, *C*). Near the center of the degenerate area was a mass of crushed, dead cells, brown intercellular and intracellular deposits, and a few distorted parenchymatous cells with discolored walls. The region adjacent to this area, and between it and the center of the root, was composed of numerous small, and a few enlarged, thin-walled cells all of which were oriented radially (pl. 3 *C*, *a*), while the adjacent tissues in the direction of the periphery consisted of many hypertrophied and a few hyperplastic cells with their long axes parallel or nearly parallel to that of the main necrotic area (pl. 3 *C*, *b*).

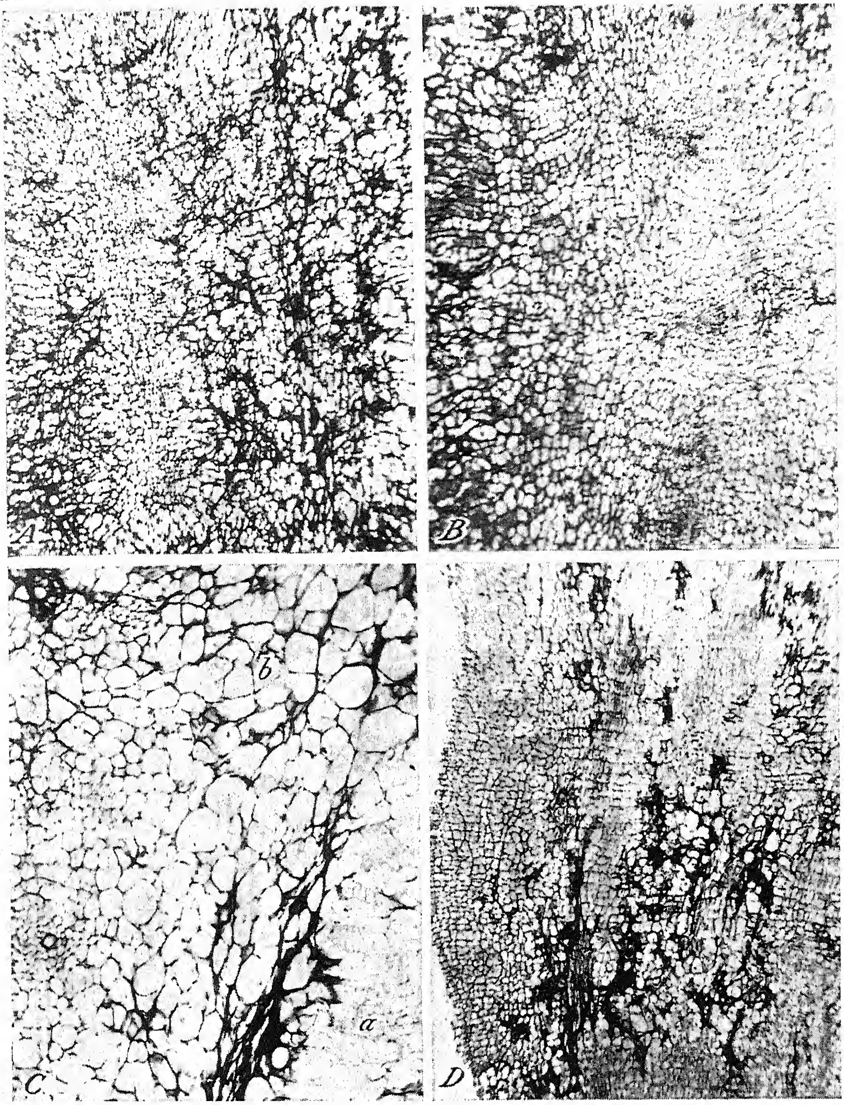
Petiole of the Leaf

True leaves in a vegetative crown differentiate successively from the apical meristem just above the cotyledonary node.

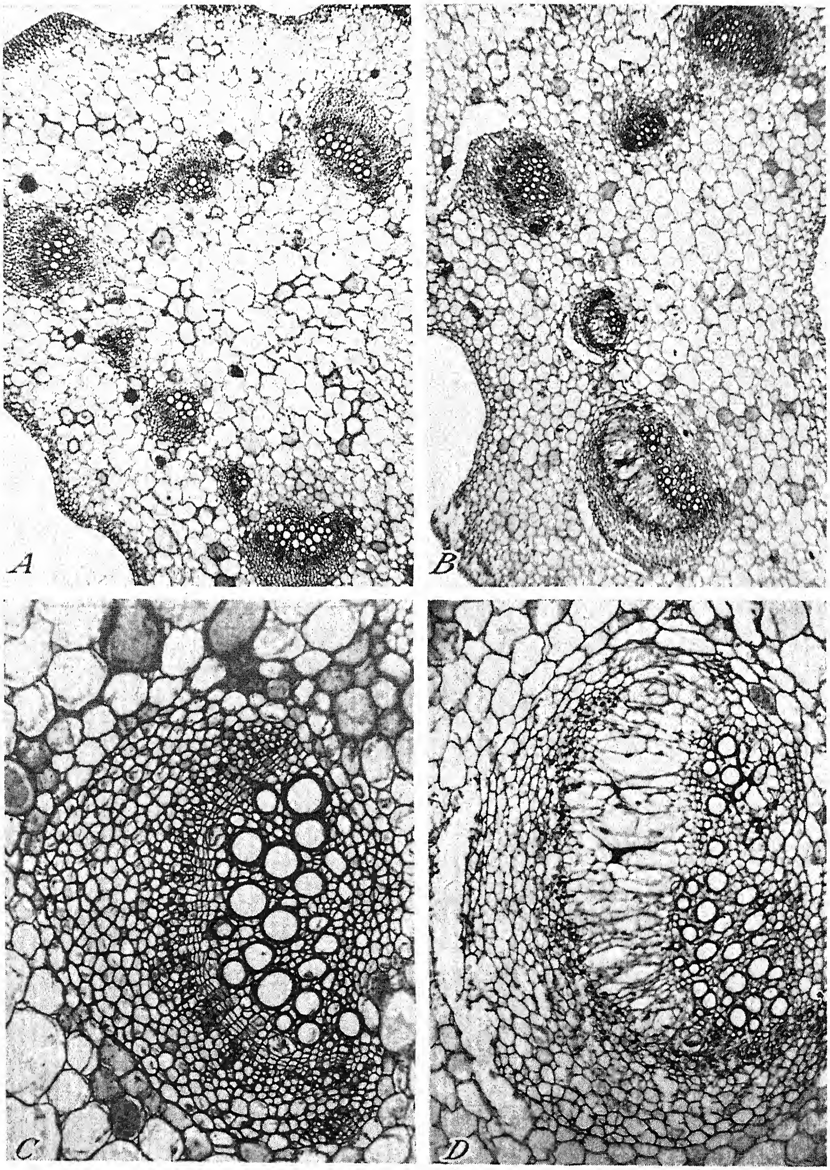
Since the newly initiated leaves of the beet develop as older ones become senescent, it was possible to obtain material at several stages of the disease from a single plant. Therefore, in histological studies, both the nature of the pathological changes induced by boron deficiency and the variation in the degree of these changes causing the different macroscopic symptoms could be determined. While the size and shape of the leaf varies among varieties, the types of tissue and their arrangement are similar in all varieties examined.

Abnormalities in older leaves were found in the petioles of old malformed leaves and in some which were macroscopically normal as well as in malformed ones (pl. 4, *D*). As in the root and hypocotyl many phloem cells contained excessive amounts of heavily stained, granular material. Frequently the cambial zone was composed of a wide band of thin-walled cells some of which were hypertrophied and others small and rapidly dividing. A few misshapen xylem elements were interspersed among the thin-walled cells. In bundles in which cell enlargement and proliferation were extensive, a few discolored cell walls and some brown deposits were present (pl. 4, *D*). In such bundles there was a decrease in the amount of normal differentiated xylem and phloem tissue. The extent of vascular disorganization varied in different bundles at a given level (pl. 4, *B*) and at different levels in the same petiole. No abnormalities were found in any other tissues in petioles of older, apparently normal leaves.

In the petioles of younger leaves which showed macroscopic symptoms degeneration appeared earlier and progressed farther. The magnitude of microscopic degeneration in these was in direct proportion to the severity of external symptoms. In the vascular bundles heavily stained phloem cells were present but were less numerous than in petioles of older leaves. A few of the younger sieve tubes and companion cells were short and irregularly shaped. The cambial zone contained many hypertrophied and a few small thin-walled cells. In some bundles a few of the giant cells extended from the xylem to the phloem. In others the cells in the cambial zone near the phloem were much larger than those adjacent to the xylem (pl. 5, *C*). Scattered among the thin-walled cells near the xylem were strands of distorted scalariform vessel segments which were oriented at various angles. Often successive elements failed to join or were only partly connected. There was seldom any evidence that these strands were



"Taproots" of boron-deficient garden beets. *A* and *B*, Adjacent transections near periphery of a young field-grown plant. Note that some vascular tissue in the severely diseased region is not disorganized; slight degeneration in one bundle of the inner ring. X 26. *C*, Transection from a taproot near maturity. Note the radial rows of proliferated cells (*a*) inside the necrotic area and the hypertrophied cells (*b*) outside the necrotic area. Note also the necrosis around a xylem element in upper left corner of photograpn. X 44. *D*, Longisection of young beet near periphery. Note the difference in the extent of degeneration at the different levels. X 18.



Transection of petioles from older leaves of garden beet. *A*, Normal petiole. X 39. *B*, Boron-deficient petiole; note the wide zone of thin-walled cells in the cambial region of the two lower bundles. X 30. *C*, Enlargement from *A*. X 145. *D*, Enlargement from *B;* note the wall discoloration in some of the hypertrophied cells. X 91.

connected with the older xylem tissues, in which only a few of the vessel segments were misshapen. In a few specimens some xylem parenchyma cells were hypertrophied.

In the more severely affected bundles there was considerable cell discoloration and disintegration in the cambial zone. Although in a few specimens the discoloration first appeared near the differentiated tissues (pl. 5, *C*), the first evidence of degeneration in most cases was a slight discoloration of adjacent walls of 2 or 3 cells, usually hypertrophied ones, in the cambial zone similar to that shown in older leaves (pl. 4, *D*). As such an area of discolored tissue enlarged, some cells near the center were crushed and discolored. Others retained their form but were darkened by heavily stained deposits. In acute cases the necrotic areas included portions of the xylem, phloem, bundle caps, starch sheath, and adjacent parenchyma as well as the cambial zone (pl. 5, *C*). In some bundles (pl. 5, *B*, *C*) there was considerable necrosis in the phloem and starch sheath, but little or none in the bundle cap between them. When a portion of the xylem bundle cap or starch sheath was involved in a necrotic area, the adjacent cells were often hypertrophied (pl. 5, *B*, *C*). Parenchyma cells between badly disorganized bundles were also much enlarged (pl. 5, *C*). In addition to degeneration in and near the vascular bundles, necrotic areas were frequently present in subepidermal tissue. These areas were most common at the petiole margin and at the ribs, regions where collenchyma would normally be present. Cells abutting the necrotic tissue were frequently misshapen and hypertrophied. In a few specimens epidermal cells were involved in the necrotic area.

Changes in petioles of very small, stunted leaves were similar to those just described, except that there were less hypertrophy and proliferation and more cell deposits in the cambial zone (pl. 5, *B*).

Examination of petioles of unilaterally developed leaves described elsewhere (*29*, *30*) revealed that the bundles on one side showed slight if any pathological change while those on the other side had undergone extensive degeneration (pl. 4, *B*). This could account for both the unilateral development of the lamina and the intensification of red color in the poorly developed portion. Degeneration and the accompanying decrease in differentiation of vascular tissue would retard leaf development and impede the movement of materials to and from the affected region and the resultant accumulation of sugars would lead to increased pigmentation. The necrosis and proliferation found in the subepidermal tissue of the petiole might well account for the external cross-hatching symptoms (pl. 5, *B*, *D*) occasionally observed in petioles of boron-deficient garden beet (*30*).

Lamina of the Leaf

In the lamina of the normal beet leaf, xylem, phloem, and cambium are found in the midrib and larger veins, but xylem and phloem or xylem only is found in the smaller veins. The single-cell-layered epidermis is composed of irregularly shaped cells. Stomata, which are present on both dorsal and ventral surfaces, are slightly more numerous on the former. The mesophyll consists of two or three layers of palisade cells on the ventral side, which grade into spongy parenchyma cells on the dorsal side. Cells of both contain numerous

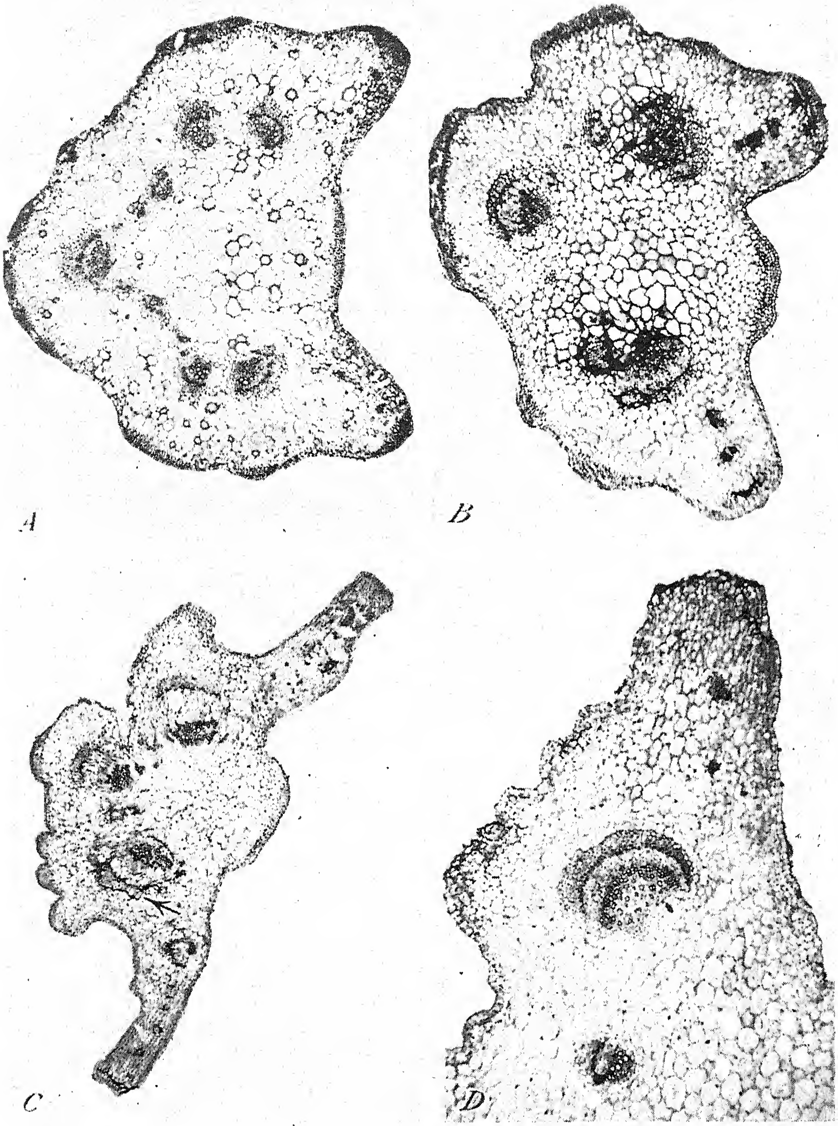
chloroplasts. It is possible that, as found by Artschwager (*2*) in sugar beet, the ratio of palisade to spongy parenchyma cells is higher in thick leaves.

In the mesophyll of the lamina of young leaves from boron-deficient plants both cell enlargement and cell proliferation occurred. Some of the hypertrophied spongy parenchyma cells were elongated with their longitudinal axis parallel to the leaf surface. The chloroplasts did not appear to be quite so numerous nor quite so green as those in normal plants. In the reddened leaves examined, the pigmentation was confined to the mesophyll cells just beneath the epidermis, except at the midrib and the larger veins. In these regions the red pigment and a brown pigment were found in two or three subepidermal cell layers. Necrotic areas were sometimes found in younger leaves, particularly near the expanding margin of the lamina. In the larger veins and in the midrib there was vascular disorganization similar to that found in the petiole.

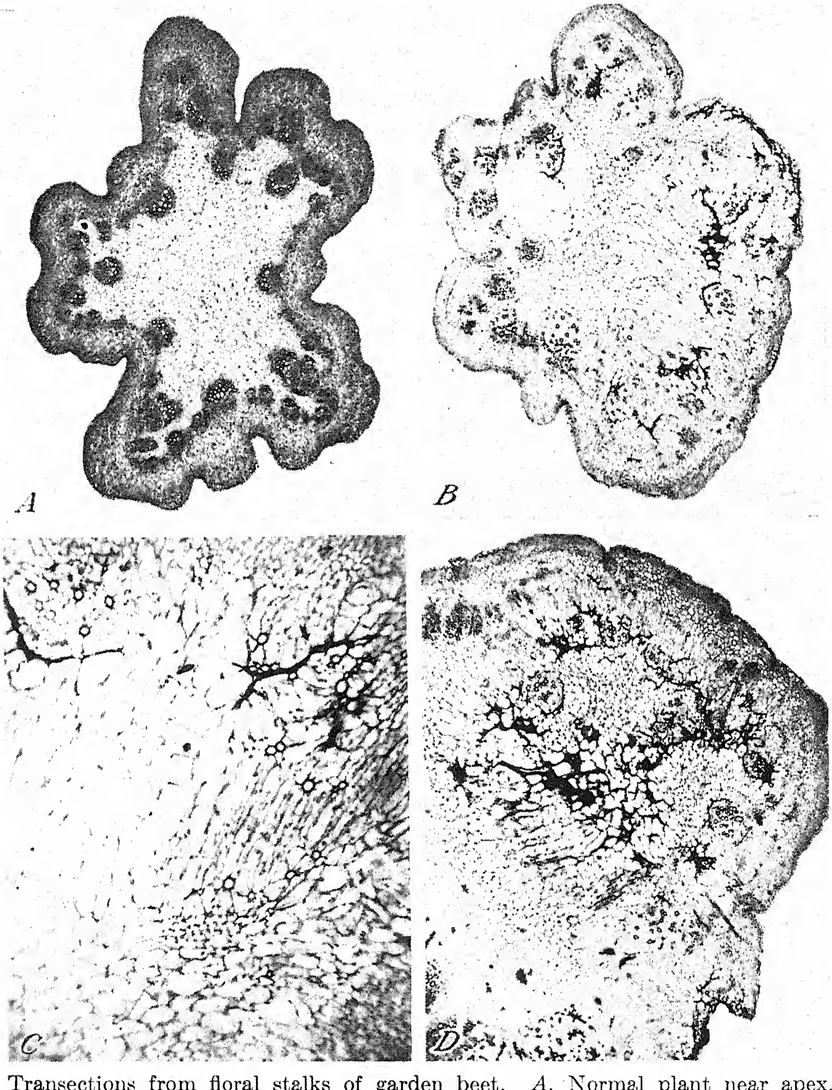
Floral Axis

As in the other organs of the plant, the changes found in the slightly differentiated tissues near the growing point of the floral axis were similar to those in the more mature tissues, except for the degree to which the various tissues were affected. In the slightly differentiated regions darkly stained phloem cells similar to those found in the root and hypocotyl were present in most bundles, but were particularly numerous in those which were otherwise quite normal. All or a portion of the cambial tissues were composed of hypertrophied and hyperplastic cells (pl. 6, *B*). The few thick-walled vessels present in the xylem were scattered among numerous, radially elongated, thin-walled cells (pl. 6, *C*). Frequently some of the parenchyma cells in the vicinity of the innermost xylem elements were crushed and heavily stained. Occasionally a xylem element had degenerated. Pith cells in large areas adjacent to such bundles were also greatly enlarged and horizontally elongated (pl. 6, *B*). Infrequently a crushed cell or a few cells with discolored walls were found in these abnormal areas. Patches of necrotic cells which were found in the subepidermal collenchyma layers occasionally extended to include epidermal cells. At some levels of the stem these necrotic areas extended inward and connected with similar areas in the vascular tissues.

In regions of the floral axis in which tertiary thickening was well under way heavily stained phloem cells were present in both secondary and tertiary phloem. The numerous thin-walled, hypertrophied cells interspersed among the primary and secondary xylem elements were not so much enlarged as similar cells near the apex (compare pl. 6, *B* and *C* with *D*). The number of already differentiated xylem elements in this region may have been a factor in checking cell enlargement. The areas of crushed cells among the xylem elements and in abutting pith tissue were larger and more numerous than in younger tissue (pl. 6, *D*). Areas of enlarged and proliferated cells found in the pith were similar to those in younger tissues except for the greater amount of necrosis. In a few cases small islands of normal parenchyma cells which extended but a few cells horizontally and vertically were found near the center of an area of abnormal pith cells. In the tertiary ring the cambial zone was often composed of a mosaic of large, radi-



Transections from garden beet petioles. *A*, Normal leaf. X 22. *B*, Boron-deficient young leaf; note extensive necrosis and accumulation of deposits in the vascular bundles, the hypertrophied cells adjacent to the two largest bundles, and the subepidermal necrosis. X 31. *C*, Boron-deficient leaf near the base of the lamina; note necrosis in phloem and starch sheath in one bundle (indicated by arrow) and necrosis in the lower wing. X 20. *D*, Portion of petiole which showed cross hatching; note abnormal areas at the top and along the dorsal margin. X 33.



Transections from floral stalks of garden beet. *A*, Normal plant near apex. X 26. *B*, Boron-deficient plant near apex; note necrosis and deposits at juncture of pith and vascular tissues and the association of pith and cortical degeneration with vascular degeneration. X 23. *C*, Enlargement from *B;* note the radial elongation of xylem parenchyma cells. X 68. *D*, Four internodes below *B;* note that hypertrophy and hyperplasia are more extensive in the region of the secondary cambium than in the xylem parenchyma. X 21.

ally elongated and small, rapidly dividing cells arranged in radial rows (pl. 6, *D*). The changes here were similar to those found in the bundles of petioles of boron-deficient beet plants. Parenchyma cells in the tertiary xylem elements were similar to those around secondary xylem elements. The areas of abnormal tissue in the subepidermal layers of collenchyma and adjacent epidermal cells varied from small, discolored cell groups to large areas in which a considerable number of cells had disintegrated. As in similar degeneration nearer the stem apex, such areas sometimes extended into and involved adjacent vascular bundles. Along the margin of the subepidermal lesions the cells were hypertrophied and intercellular deposits accumulated. Farther away from the lesion small groups of proliferated cells were interspersed among the enlarged cortical cells.

PATHOLOGICAL HISTOLOGY OF BORON-DEFICIENT CABBAGE

The macroscopic symptoms of the boron-deficiency disease in cabbage seedlings grown in sand-nutrient culture and in older plants growing in the field have been described by Walker, McLean, and Jolivette (*31*). Marked histological abnormalities were found in both seedlings and headed plants of cabbage. In seedlings these were confined for the most part to vascular tissue except near the apical meristem and in expanding leaves. In plants grown in complete solution for 2 or 3 months before being placed in minus-boron solution and in those grown on boron-deficient soils, degeneration in the stem was confined to the pith, although the changes in the younger leaves were similar to those in comparable leaves in seedlings. In view of the differences in tissues most affected in seedlings and older plants they will be considered separately. The normal histology of root, hypocotyl, and stem of cabbage has already been described by Smith and Walker (*27*), Larson (*16*), and Havis (*13*).

Root, Hypocotyl, and Stem of Young Cabbage Plants

In young plants the disorganization in the vascular system of boron-deficient plants was similar in root, hypocotyl, and stem. In all or part of the vascular ring the normal tabular cells were absent from the cambium. In their place was a zone of hyperplastic and hypertrophied cells, most of which were thin-walled and radially elongated (pl. 7, *B*, *D*). In some plants the cambial zone became several times the width of the normal vascular ring in the stem, and the entire ring at a given level was involved (pl. 7, *B*). In others abnormalities were present in only a portion of the vascular ring and the affected tissue was less hypertrophied. Frequently groups of discolored and crushed undifferentiated cells were found in the abnormal cambial zone, particularly near the xylem or phloem (pl. 7, *B*, *D*). The first sign of such degeneration was a slight discoloration of adjacent walls of two or three cells and occasional brown intercellular deposits. At later stages the degenerated areas became quite large. In the center of the large areas the cells were discolored or crushed and more of the brown deposit had accumulated. Near the margin, cells were only slightly discolored. The proportion of necrotic and crushed cells to enlarged and proliferated cells was often high when only a part of the bundles was affected.

Small groups of misshapen xylem elements were scattered throughout the major part of the abnormal cambial zone (pl. 7, *D*, and 8, *A*). This suggests that much of the undifferentiated tissue was potentially xylem in nature. In some specimens a thin layer of small cells with dense contents was present near the differentiated phloem (pl. 7, *B*). The fact that a few distorted phloem groups but no abnormal vessel segments were found outside this layer suggested that it was a nonfunctioning cambium.

In boron-deficient plants there was less differentiated xylem and phloem tissue than in normal plants, and what there was tended to mature earlier. This was particularly noticeable in the early lignification in the xylem and in pericycle fibers. A similar tendency for some cells to mature early in boron-deficient plants was noted in pea roots by Sommer and Sorokin (*28*).

In the root and hypocotyl, a condition of hypertrophy, hyperplasia, and necrosis similar to that described for the vascular system was sometimes found in the periderm (pl. 7, *D*).

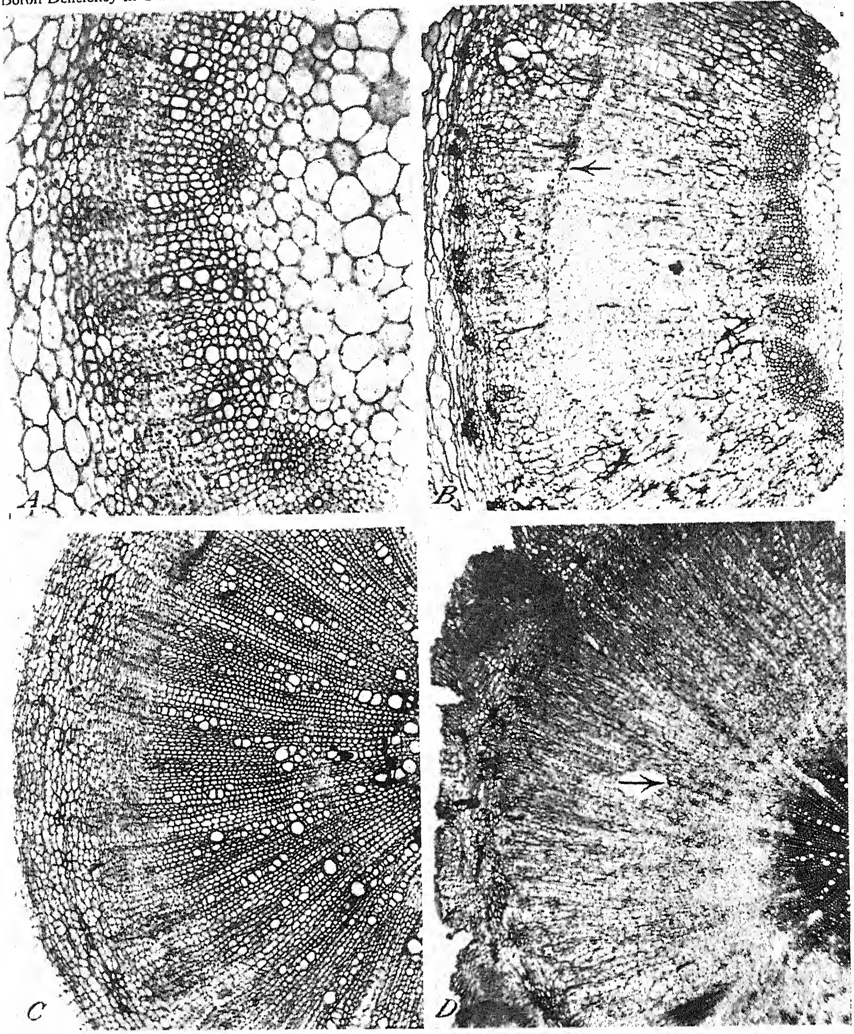
Near the stem apex, abnormalities were sometimes found in the cortex and epidermis in addition to those in the vascular tissues. The affected areas in the cortical parenchyma contained enlarged cells and proliferated cells. Some brown deposits were present among the cells. In late stages some of the affected cells disintegrated.

In the cortical, ray, and pith parenchyma cells of the remainder of the stem, the hypocotyl, and the root, starch accumulation, which may have been a direct or indirect effect of boron starvation, was noticed. This symptom was most noticeable in plants grown in the complete nutrient solution for 3 or 4 weeks before being placed in minus-boron solution, and was sometimes found when no other microscopic symptoms were noted. In all cases little or no starch was found in comparable normal plants fixed at the same time as the boron-deficient plants.

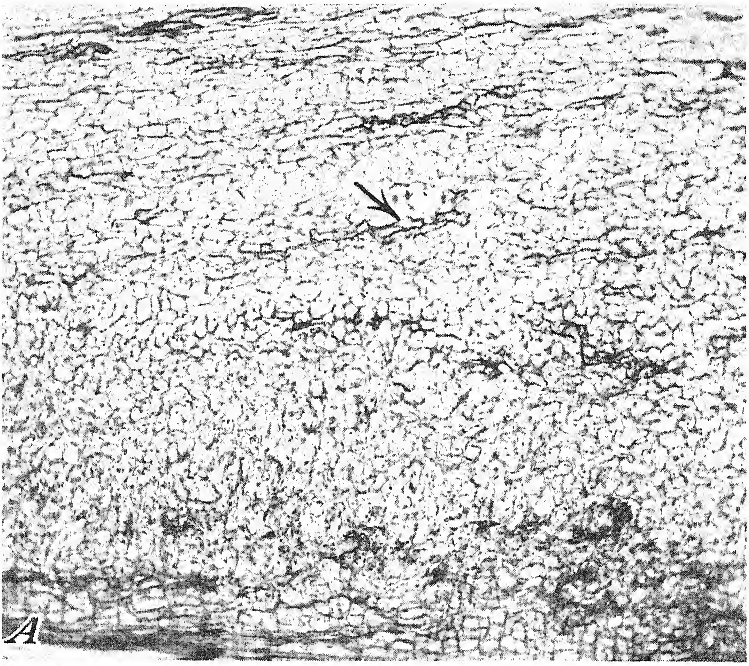
Leaves of Young Plants

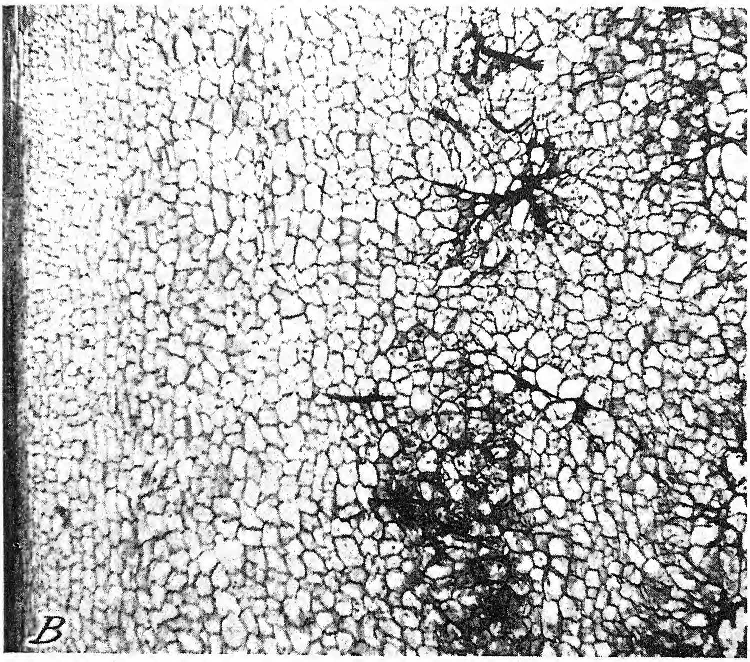
When plants were grown from seed in minus-boron, sand nutrient cultures, the few leaves that developed were affected very early and therefore the effect of boron starvation could be studied on very young tissues only. However, when plants were supplied with complete solution for several weeks before being transferred to minus-boron cultures, successive leaves were at different stages of development when the supply of boron became inadequate. In these it was possible to compare the nature and extent of degeneration caused by boron deficiency in very young leaves with that in leaves at various stages of development.

The most extensive degeneration in the leaf petiole was found in the vascular tissue. While abnormalities varied in magnitude with the age of the leaf, they were in nature similar at various ages. Most bundles in the larger vascular strands were not entirely separated by parenchyma rays as in normal petioles. Instead their xylem elements were merged into one solid core or arc. In extreme cases there was considerable horizontal elongation of the thin-walled cells among the differentiated xylem elements. Usually instead of a normal cambium between the layer of differentiated xylem and the phloem of each of the several bundles there was a zone of misshapen cells similar to



Transections from young cabbage plants. *A*, Normal stem. X 65. *B*, Boron-deficient stem; note layer of small cells suggestive of a degenerated cambium (indicated by arrow) and the small necrotic areas scattered through the zone of thin-walled cells in the cambial zone. X 33. *C*, Normal root. X 47. *D*, Boron-deficient root; note the thick-walled xylem elements scattered in the abnormal tissue as indicated by arrow. X 23.





Longisections from boron-starved cabbage. *A*, Hypocotyl of a young plant; note the distorted xylem elements (indicated by arrow) scattered in the undifferentiated tissue. X 53. *B*, Stem of plant approaching head stage; note that although there is evidence of cell division and enlargement near the vascular tissue at left, degeneration is confined to the central part of the pith. X 19.

but not so extensive as that found in the root and stem (pl. 9, *D*). In some specimens the hypertrophy of the cells near the xylem was greater than that in other cells in the undifferentiated zone. Frequently small areas of necrotic cells were found in the undifferentiated tissue (pl. 9, *D*). Occasionally a few misshapen xylem elements were found in the inner portion of the undifferentiated zone. In such cases the necrotic areas usually centered about these elements. As in the root and stem, a layer of heavily stained cells, which appeared to be an abnormal cambial layer, was sometimes discernible in this area located a few cells inward from the differentiated phloem. A few misshapen degenerated cells resembling sieve tubes were found adjacent to the normal differentiated phloem. In the larger vascular strands the phloem of each bundle was usually separated from that of neighboring bundles by radial bands of parenchyma.

In the cortical parenchyma starch grains were numerous. In regions where cross hatching occurred, necrotic and proliferated cell groups were found involving subepidermal and, to a lesser extent, epidermal tissues.

As was found in the garden beet, degeneration occurred in some of the older, apparently normal leaves. In plants with macroscopic symptoms the histological changes occupied larger areas than in those in which symptoms were not visible.

In a study of a limited amount of leaf-lamina material, changes similar to those in the petiole were found in the vascular tissue. In thickened leaves of boron-deficient plants there was an increase in the size of some cells and in the number of cells present in the mesophyll. In it the cells appeared to be packed more tightly than in normal tissues. Chloroplasts were equal in size or slightly larger than those in normal plants.

Older Plants

The normal anatomy of plants grown for 3 or 4 months in complete nutrient solution and of plants transplanted into the field was similar to that of several-week-old seedlings except that more secondary tissue was present in the vascular ring and more pith in the stem of the older plants and the cortex was wider. In some specimens a 2- or 3-mm. core of tissue in the center of the pith was water-soaked or green. Cells in this area, however, showed no evidence of degeneration.

When the boron supply became inadequate for older plants which had not yet headed, symptoms similar to those in leaves of boron-starved younger plants were sometimes present in the outer leaves. However, the major degeneration, internal necrosis, appeared in the central part of the pith throughout the stem and upper hypocotyl, regions in which no symptoms except starch accumulation appeared in younger cabbage plants. In studies of a limited number of larger roots of older plants, no apparent abnormalities were found. For this reason histological studies on that portion of the plant have been left for a later investigation.

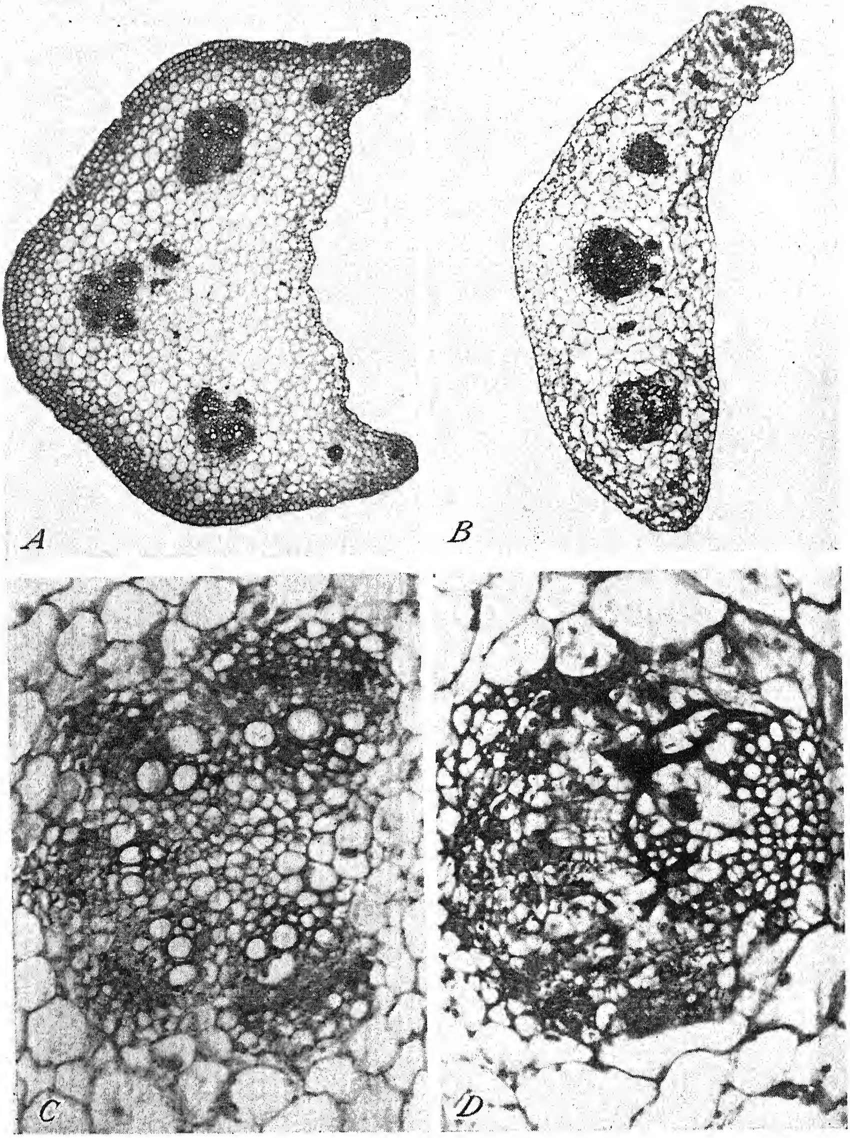
In the pith microscopic changes involved a slightly larger area than the macroscopically visible symptoms. In plants transferred to a boron-deficient solution after having grown in complete solution for 2 or 3 months, a greater proportion of the pith was involved than in normal plants transplanted into boron-deficient fields. Nevertheless,

the changes were the same except that there were fewer cells with thickened walls in the pith of the plants grown in sand culture (p. 8, *B*, and 10, *B*). At and near the margin of the affected pith, isolated groups of abnormal cells were found, while near the center the majority of the cells were abnormal (pl. 10., *B*). The marginal cell groups usually occupied an area which was roughly spherical. These areas contained several types of abnormal tissue arranged in a fairly consistent pattern (pl. 10, *C*, *D*). In the center of the degenerated tissue were thick-walled cells which sometimes contained deposits of a brown material. In larger areas some of these cells were crushed or disintegrated. Scattered among or surrounding these was a zone of large cells some of which had partly thickened walls. Others had thin cross walls. Such cells were similar to those first described except that degeneration had not progressed so far. The size of this zone varied but was roughly proportional to the size of the affected area. The walls of most of these cells and of the other thick-walled ones in the abnormal tissue took safranine when the safranine and fast-green stain were applied. All other pith cells took fast green.

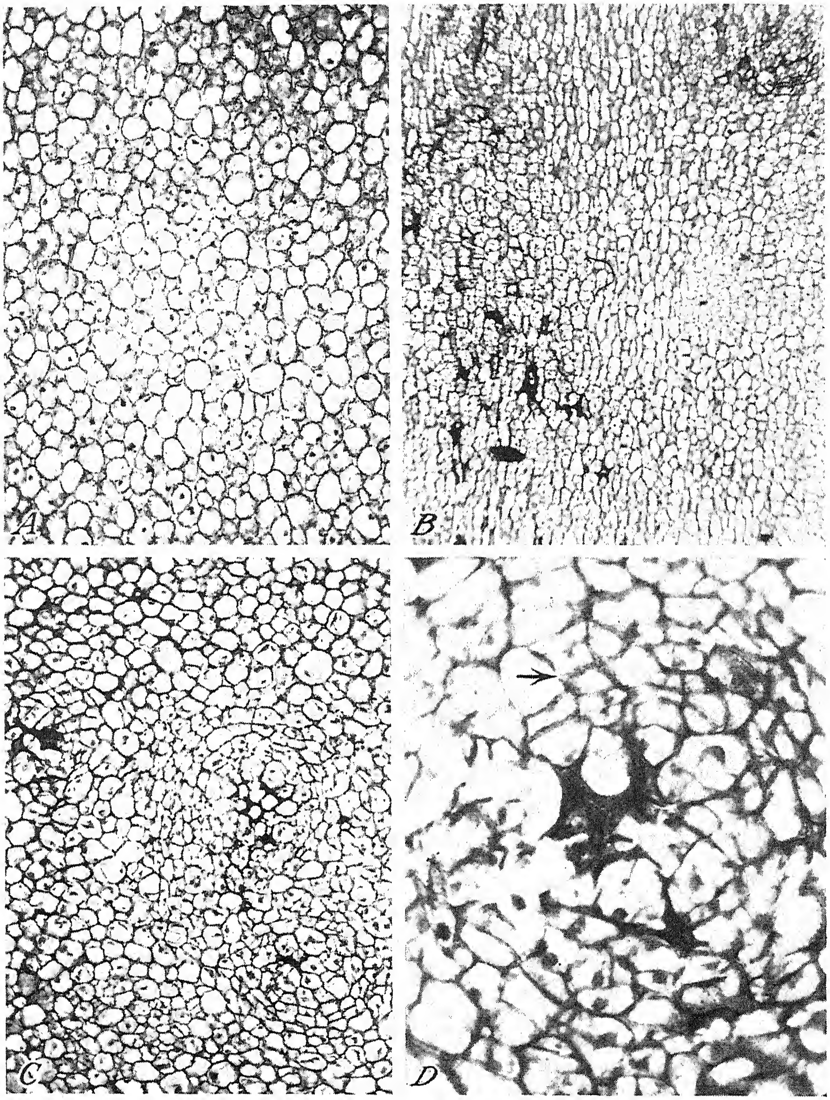
Surrounding the zone of large cells in the more extensive degenerate areas was one of small, thin-walled, rapidly dividing cells which in some cases entirely walled off the inner tissue. Intermingled with and surrounding this layer was a one- or two-cell zone of sclerotic cells (pl. 10, *D*) which had scalariform, reticulate, or pitted walls. Proliferation sometimes occurred just outside this zone, but the daughter cells were nearer normal size than in the hyperplastic tissue near the center of the disorganized area. Some of the isolated abnormal cell groups later coalesced. In such cases it was difficult to make out distinct patterns. In the central portion of affected pith tissue the several types of cells mentioned were intermingled in random fashion (pl. 10, *B*). It is probable that such a case represented several coalesced groups of abnormal cells. Occasionally islands of normal parenchyma cells, similar to those in the pith of the floral stem of garden beet, were found.

DISCUSSION

The pathological changes in boron-deficient cabbage and garden beet have many points in common. The greatest degree of break-down in beets at all stages and in cabbage in early development was found in those regions in which cell division and tissue differentiation were most active. The reactions appeared to be intimately associated with cell metabolism rather than with a particular tissue. In beet seedlings in which secondary thickening had just begun the principal break-down was in phloem cells. In those in which tertiary thickening had begun degeneration was in the xylem as well as in the phloem of differentiated rings. In somewhat older "taproots" most necrosis was found in rings in which early differentiation was under way, less in the next older rings, and none in the oldest rings. In young cabbage plants the chief abnormalities in root and stem were also found in the differentiating vascular tissues. The greatest difference in the reaction of cabbage as compared with that of beet was found in the older plants in which the head had developed. Even though there was still meristematic activity in the vascular tissue, disorganization was confined almost entirely to the central portion of the pith.



Transections from cabbage petioles. *A*, Normal petiole. X 30. *B*, Boron-deficient petiole. X 24. *C*, Enlargement from *A*. X 140. *D*, Enlargement from *B;* note the abnormal cells and necrotic areas in the undifferentiated tissue and the position of the differentiated xylem as compared to that in *C*. X 144.



Central part of pith of headed cabbage. *A*, Transection from a normal plant. X 35. *B*, Longisection from a boron-deficient plant; note the thick-walled cells at lower margin of small degenerate area in upper right of photograph and proliferation and deposit accumulation in tissue at left of photograph. X 21. *C*, Transection from boron-deficient plant at margin of degenerate pith area. X 28. *D*, Small degenerate area adjacent to *C;* note the cross walls in cells in the center of the area and the wall thickenings (indicated by arrow) in the surrounding ring of cells. X 120.

It was evident in roots, stems, and leaves of both species that microscopic abnormal development occurred in advance of macroscopic symptoms and that the former was usually more extensive than could be recognized macroscopically. It was also to be noted that microscopic changes occurred which did not progress to the point where macroscopic symptoms became visible. Sudden severe manifestations of disease thus may often have been preceded by slow, protracted invisible degeneration. Factors such as increased moisture and temperature favorable for sudden increases in growth rates of these plants would then contribute to a sudden appearance of extensive visible disease symptoms.

The details of histological changes, though often varied in their grosser macroscopic manifestations, usually followed a fairly uniform pattern. Cell wall discoloration, hyperplasia, and hypertrophy were all common initial cellular reactions in thin-walled differentiating tissue. As death of a group of cells occurred, increased cell division or cell growth commonly followed in the vicinity of the dead cells. This often led to islands of pathologic tissue as noted in the pith of maturing cabbage. Where continued cell activity predominated, as in beet roots and young cabbage stems, normal differentiation of vascular elements was checked. In other cases abnormal differentiation of thick-walled cells appeared as in cabbage-pith lesions. It is thus evident that the fundamental effect of boron deficiency in both species is one of disturbance of growth-regulation processes, while the course of events following the initial response varies with the tissues concerned.

The picture of pathological histology indicates strongly that boron is particularly important in the metabolic processes concerned in cell division and tissue differentiation. It may also be of peculiar value to the development of storage tissue. One of the physiological characteristics of boron pointed out previously (*30*) is its ready mobility in the plant when first introduced and its tendency to become immobile rather rapidly in the tissue.

The first pathologic effects of boron deficiency, then, may be considered physiological, exhibited locally by disturbance of growth relations and manifested by both hypertrophic and hyperplastic cell reaction. Necrosis follows and byproducts released from dead or dying cells may then also become factors influencing cell growth. General physiological effects follow through disturbed tissue differentiation and function. Abnormal organization of the xylem and phloem with reduced amounts of elements adapted to translocation leads to an accumulation of sugars in developing beet leaves and consequent excessive formation of pigment. Further vascular dislocation leads to stunted growth in tissues normally supplied by the xylem and phloem concerned. Thus boron deficiency commonly leads to such general effects as stunting, malformation of organs, unilateral growth, and death. Furthermore, mild shortage of boron insufficient to produce macroscopic symptoms may lead only to microscopic injury to transporting tissue. It is in such instances that the application of borax to growing crops commonly results in increased growth, which is reflected in greater yields, even though deficiency symptoms do not appear in untreated controls (*30*).

SUMMARY

This investigation comprises a study of histological changes which occur in garden beet and cabbage grown with a deficiency of boron in the nutrient supplied to greenhouse sand culture, and under natural conditions in the field.

In beet root and hypocotyl the first sign of disease in young seedlings is in the contents of certain phloem cells and occasional hypertrophy of cambial cells. In slightly older plants degeneration appears in primary and secondary xylem as intercellular brown deposits, accompanied by distortion of cells. Groups of disintegrated cells are frequently surrounded by a border of proliferating cells which may involve one or more bundles, thus giving rise eventually to macroscopic necrotic areas. In roots which are somewhat older when the deficiency of boron becomes acute, the most severe necrosis occurs in the tertiary ring or rings most active in differentiation at the time.

Abnormal development in beet leaves also centers in the bundles of the petiole. It is found commonly in old leaves which were normal macroscopically and in which it has occurred too late to influence gross morphology. In younger leaves the magnitude of histological change is in direct proportion to the severity of external symptoms. In petioles of unilaterally developed leaves the bundles on one side show slight, if any, pathological change while those on the other side have undergone extensive degeneration. Cell enlargement and proliferation extend to the mesophyll and spongy parenchyma of the leaf lamina.

In the floral axis, proliferation of cells occurs in the cambium and in the pith. Necrotic areas occur in the pith and in the vascular region of secondary and tertiary rings and are sometimes connected with similar regions in the cortex, where the lesion occasionally involved epidermal cells.

In young cabbage plants extensive proliferation of cells in the region of the cambium of root, hypocotyl, and stem results in a band of meristematic tissue several times the usual width of the undifferentiated zone between phloem and xylem. There is correspondingly less differentiation of vessels and phloem elements. Necrotic areas develop in this undifferentiated area. Abnormalities are to be seen occasionally in the cortex of the stem apex but necrosis in the pith is rare until the plant has approached the head stage. When boron deficiency symptoms appear in the field they are usually confined to this region. In cabbage leaves disturbances in differentiation of the vascular bundles in petiole and lamina are the chief evidences of the disease.

In both species the shortage of boron appears to affect chiefly the metabolic processes concerned in cell division, cell differentiation, and possibly storage. The initial reaction of cells is seen in cell-wall discoloration, discoloration of cell contents, excessive cell division, and abnormal enlargement. Necrotic areas which follow are made up of disintegrated cells which are often surrounded by a region of abnormally active tissue consisting of hyperplastic cells, hypertrophied cells, and sometimes peculiarly differentiated ones.

The pathological histology of internal black spot of garden beet and internal breakdown in the pith of cabbage is the same in nutrient-grown and field-grown plants. In both species extensive histological changes occur before macroscopic symptoms appear.

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DIFFERENTIAL EFFECT OF NUTRIENT SOLUTIONS ON THE SIZE OF VARIOUS PARTS OF MAIZE SEEDLINGS GROWN IN THE DARK [1]

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INTRODUCTION

The American species of grasses belonging to the maize tribe (Tripsaceae) and at least one oriental member of the tribe, *Coix lacryma-jobi* L., have greatly elongated mesocotyls (epicotyls) when their seedlings are grown in the dark under suitable conditions of moisture and temperature.

With the object of utilizing the very open scale afforded by the mesocotyl as a measure of the growth substance and hence possibly the growth potentialities of the plant, a long series of experiments has been conducted to determine the effect of temperature and of various radiations of known wave length and energy upon the elongation of the mesocotyl.

Difficulties were encountered in producing uniform effects with given treatments, and it was soon found that for comparative results there must be a rigid control of all environmental conditions. Slight variation in temperature, above or below the optimum, produced measurable effects and, in the case of wave-length studies, slight variations in energy resulted in detectable responses.

In these studies the seedlings were grown in sterilized sand which, with washing and sterilizing, was used repeatedly. Lack of agreement between repeated experiments and excessive variability of subgroups within experiments compelled increasing refinements in control of conditions of growth and led eventually to a study of the effect of the solution upon developing seedlings.

In considering the effect of culture solutions, it should be kept clearly in mind that, although both cell expansion and division are involved, there is no growth in the sense of an increase in dry matter over that supplied by the material stored in the seed. The seedlings were in total darkness from the time of planting until they were finally measured, and therefore there was no photosynthesis. The experiments reported here were concerned only with those processes that ordinarily take place before the plumule reaches the soil surface.

ELONGATION OF MESOCOTYL

EFFECT OF TAP WATER AND COMPLETE CULTURE SOLUTION AT VARIABLE TEMPERATURE

The first test of the effect of the culture solution was a comparison of the length of mesocotyl obtained in tap water with that produced by an equal quantity of Eaton's (*3*) [2] solution modified by increasing the monopotassium phosphate (KH_2PO_4) from 3 to 30 gm. for 100

[1] Received for publication March 27, 1942.
[2] Italic numbers in parentheses refer to Literature Cited, p. —.

liters. This experiment involved 40 cans, each containing 520 gm. of oven-dry sand moistened with 110 cc. of solution or tap water. Each can was planted with 15 seeds of Funk Yellow Dent Corn (*Zea mays* L.). The entire set was arranged in a space of 2 by 3 feet in 5 blocks, each containing 4 replications of each solution. The replications within blocks were paired, randomized as to position within and between blocks. The entire set was grown in an air-conditioned room where the maximum temperature had an upper limit at 80° F. but the minimum temperature was permitted to drop to 70°. The seeds were planted September 28, and the plants were measured October 5, 1939. Only the lengths of mesocotyls were recorded. The mean length for plants grown in tap water was 99.61±3.35 mm. and for those grown in the culture solution 124.75±3.41 mm. The variance, apportioned on the basis of individual plant measurements, is shown in table 1.

TABLE 1.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square	Source of variation	Degrees of freedom	Mean square
Total	575	3, 584. 59	Within subgroups	15	4, 810. 19
2-can groups	19	[1] 7, 330. 82	Solution	1	[1] 90, 969. 45
8-can subgroups	4	[1] 16, 783. 20	Error	555	3, 298. 87

[1] $P<0.01$.

From this analysis it is evident that the culture solutions were associated with differences in length of mesocotyl.

EFFECT OF TAP WATER AND FIVE SALT SOLUTIONS AT VARIABLE TEMPERATURE

The second test of the effect of culture solutions involved six solutions, one being tap water and the others as follows:

	Grams per 100 liters
Complete solution:[1]	
$Ca(NO_3)_2+4H_2O$	94
KNO_3	30
$(NH_4)_2SO_4$	27
$MgSO_4+7H_2O$	49
KH_2PO_4	30
No potassium:	
$Ca(NO_3)_2+4H_2O$	163. 8
$(NH_4)_2SO_4$	26. 4
$MgSO_4+7H_2O$	49. 0
$Na_2HPO_4+12H_2O$	68. 4
No magnesium:	
$Ca(NO_3)_2+4H_2O$	93. 6
$(NH_4)_2SO_4$	26. 4
No magnesium—Continued.	
KNO_3	30. 3
KH_2PO_4	27. 4
$CaSO_4+2H_2O$	34. 4
No calcium:	
$(NH_4)_2SO_4$	26. 4
KNO_3	70. 7
KH_2PO_4	27. 4
$MgSO_4+7H_2O$	48. 8
No ammonium:	
$Ca(NO_3)_2+4H_2O$	117. 0
KNO_3	50. 5
$MgSO_4+7H_2O$	48. 8
KH_2PO_4	13. 7

[1] Eaton's solution modified.

In all these solutions the minor salts, as given by Eaton, were added. The experiment was set up with 96 cans, each containing 520 gm. of sterilized oven-dry sand moistened with 120 gm. of solution and planted with 15 seeds of Funk Yellow Dent corn. The cans were arranged in 16 blocks, each block containing 1 can of each solution. The arrangement of the solutions within the block was random. The experiment was located in the air-conditioned room used in the first test and the entire block of cans was covered with a tarpaulin to minimize wanton air currents that might produce variable temperature. The cans were planted December 1, and measurements of mesocotyl length were made on December 11, 1939.

The apportioned variance on the basis of individual plants is given in table 2.

TABLE 2.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square
Total	1,374	1,582.90
Replicates	15	1,108.45
Solutions	5	[1] 10,920.92
Error	1,354	1,555.29

[1] $P<0.01$.

Obviously the culture solutions were associated with differences in mesocotyl length. The mean length of mesocotyl for each of the six cultures is given in the following tabulation.

Solution:	Mean length of mesocotyls (mm.) [1]	Solution—Continued.	Mean length of mesocotyls (mm.) [1]
Tap water	154.77	No magnesium	167.64
Complete solution	164.44	No calcium	156.11
No potassium	161.95	No ammonium	172.92

[1] Differences between means less than 7.56 mm. are not significant.

The mesocotyls produced in tap water are significantly shorter than those from any culture except that lacking calcium. The complete solution and the solutions lacking potassium or magnesium produced mesocotyls of comparable length, but omitting ammonium sulfate resulted in a definite increase in length of mesocotyl. The mesocotyls in this test were much longer than those of the first test, but this second experiment ran 3 days longer.

EFFECT OF TAP WATER AND SEVEN SALT SOLUTIONS AT VARIABLE TEMPERATURE

The third experiment repeated the second, with the addition of two solutions, one lacking both calcium and ammonium, the other containing only calcium salts. The composition of these new solutions was as follows:

No calcium or ammonium:	Grams per 100 liters	All calcium:	Grams per 100 liters
$NaNO_3$	45.5	$CaSO_4+2H_2O$	103.8
KNO_3	50.5	$Ca(NO_3)_2+4H_2O$	163.8
KH_2PO_4	13.7		
$MgSO_4+7H_2O$	48.8		

The experiment was set up in 96 cans, 12 for each of the 8 culture solutions. Each can contained 600 gm. of oven-dry sand moistened with 120 cc. of solution and planted with 20 seeds of Funk Yellow Dent corn. The cans were arranged in 12 groups, each group containing 1 member of each solution. The cans within groups were randomized, as were the positions of the groups. Seeds were planted December 28, 1939, and the experiment was terminated January 8, 1940. The location was identical with that of the previous experiment.

The apportioned variance on the basis of individual plants is given in table 3.

TABLE 3.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square
Total	1,848	1,747.82
Replicates	11	1,351.33
Solution	7	[1] 4,930.95
Error	1,830	1,738.03

[1] $P<0.01$.

The effect produced by the solutions, although not as pronounced as in the previous test, is clearly significant. The mean length for each of the eight cultures is given in the following tabulation:

Solution:	*Mean length of mesocotyl (mm)*[1]	Solution—Continued.	*Mean length of mesocotyl (mm.)*[1]
Tap water	170.16	No calcium	168.45
Complete solution	177.72	No ammonium	175.92
No potassium	172.19	No calcium or ammonium	165.87
No magnesium	175.72	All calcium	178.59

[1] Differences between means less than 7.78 mm. are not significant.

It is evident that the maximum elongation of the mesocotyl is dependent upon calcium and is not greatly affected by any one of the other salts in the solutions used. The differences in length of mesocotyl between this test and the preceding one can be charged only to the temperature variation, as the growing period in both cases was 11 days.

ELONGATION AND DRY WEIGHT OF SEEDLING PARTS

EFFECT OF DISTILLED WATER, TAP WATER, AND THREE SALT SOLUTIONS AT TWO CONSTANT TEMPERATURES

The evident interaction of temperature and solution made it desirable to test the effect of the culture solutions under different temperatures and with more rigid temperature controls.

Two chambers were available, operating at uniform temperatures, one at 72°±1° F., the other at 85°±2°. These two temperatures are about equidistant from the optimum of 78° found for mesocotyl elongation.

Five cultures were used, namely, distilled water, tap water, Eaton's solution as given on page 184, this same solution at doubled concentration, and the calcium solution given on page 185. The seeds were planted in cans holding 600 gm. of oven-dry sand moistened with 120 cc. of solution. Ten seeds were planted in each can through a perforated disk resting on the surface. This latter was used to permit excising the seedlings at the surface free from sand so that dry weights could be obtained. The total lengths of mesocotyls, therefore, as given in this test are shorter by 2 cm. more or less than their actual lengths because of the section left in the sand.

Ten cans of each culture were planted at each temperature and randomized within temperatures. They were not disturbed from the time of planting until the plants were measured. The series at 72° F. was grown for 12 days and that at 85° for 10 days.

Three measurements of length were made on each seedling, namely, length of mesocotyl, coleoptile, and leaves. The sum of the lengths of mesocotyl and leaves gives the total seedling height. Oven-dry weights of the total product of each can were obtained separately for the mesocotyls and for the leaves, including the coleoptiles.

In order to eliminate unequal frequencies, only the means of cans were used in analyzing the data, as germination was not perfect

The variance was apportioned for each of the three measurements of length and for three measurements of weight, namely, weight of mesocotyl, weight of leaves and coleoptile, and weight per meter of mesocotyl.

The means for the several seedling parts in the different cultures at the two temperatures are given in table 4.

TABLE 4.—*Mean lengths and weights of seedlings and parts of seedlings grown in the dark in various media and at different temperatures*

Culture	Mean length, at temperature shown (° F.), of—								Mean oven-dry weight, at temperature shown (° F.), of—							
	Mesocotyl		Coleoptile		Leaves		Seedling		Mesocotyl		Leaves		Weight per meter of mesocotyl		Seedling	
	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Distilled water	114.9	145.2	55.4	56.6	144.7	135.4	259.6	280.6	0.0171	0.0153	0.0306	0.0265	0.149	0.105	0.0477	0.0418
Tap water	120.2	140.5	55.2	56.4	161.9	147.6	282.2	288.1	.0186	.0158	.0359	.0269	.154	.113	.0545	.0427
Eaton's	121.3	139.5	61.9	61.0	191.2	182.7	312.4	322.2	.0156	.0130	.0427	.0346	.129	.093	.0583	.0476
Eaton's × 2	134.0	152.7	72.3	68.1	201.9	183.2	335.9	335.8	.0155	.0150	.0506	.0409	.116	.099	.0660	.0559
Calcium	129.9	145.6	59.0	58.6	154.1	141.9	284.0	287.5	.0154	.0139	.0335	.0256	.118	.095	.0491	.0396
Difference required for significance (P=0.01)	13.48		4.84		25.4		23.6		.0030		.0052		.0150		.004	

The measurements and figures 1 to 8 show that the temperatures were only partly equalized by the time differential. The analyses of variance (table 5) show significant temperature effects for all measures other than length of leaves and coleoptiles. The only significant interaction of culture with temperature is that for the weight per meter of mesocotyl. The mesocotyls grown in salt solution at 72° F. are relatively lighter in weight per unit length than those grown in the same solutions at 85°.

TABLE 5.—*Analyses of variance based on means of 120 cans of 10 seedlings for the seedling parts shown*

Source of variation	Degrees of freedom	Mean square					
		Length			Weight		
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Per meter of mesocotyl length
		Mm.	*Mm.*	*Mm.*	*Gm.*	*Gm.*	*Gm.*
Total	119	323.84	53.39	1,309.31	0.00001	0.00009	0.00060
Temperature	1	[1] 11,197.29	14.62	1,894.09	[1] .00007	[1] .00151	[1] .02600
Culture	5	[2] 611.37	[1] 608.68	[1] 12,760.55	[1] .00003	[1] .00109	[1] .00300
Interaction	5	220.07	20.55	566.76	.00001	.00003	[1] .00100
Error	108	214.65	29.56	808.13	.00001	.00003	.00028

[1] $P<0.01$.
[2] $P<0.05$.

Interactions of temperature and culture could be expected in this experiment only if the 2 days' difference in growth period allowed between the two temperature groups failed to equalize the temperature differential. The only possibility for a temperature effect independent of time would be in case of threshold effects, and these could hardly be expected with the temperature range used.

The entire experiment was somewhat disappointing in efficiency, since differences between pairs of means of less than 10 percent could not be established for most measurements. In the case of the weight of mesocotyl per unit length, differences of less than 20 percent between means of treatments are of questionable validity.

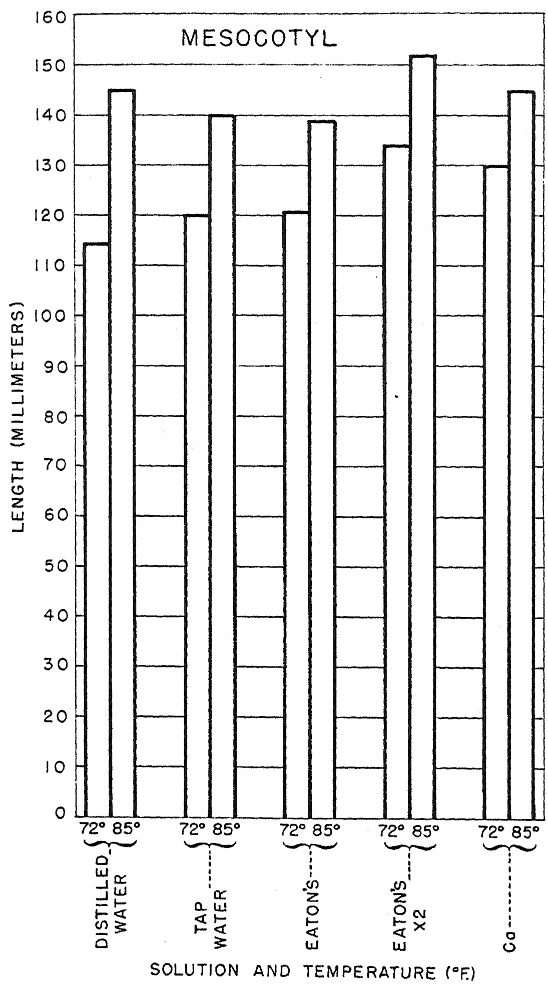

FIGURE 1.—Mean length of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

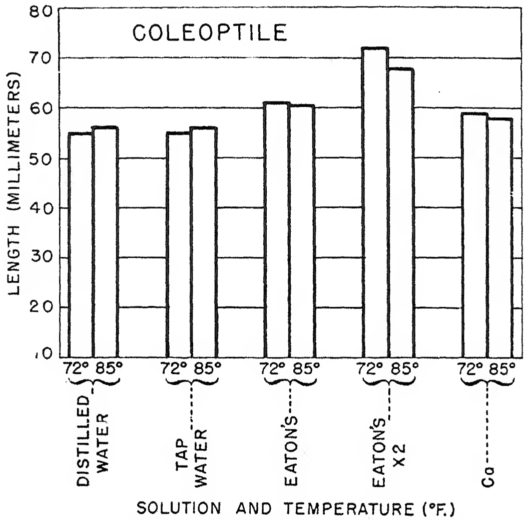

FIGURE 2.—Mean length of coleoptiles of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

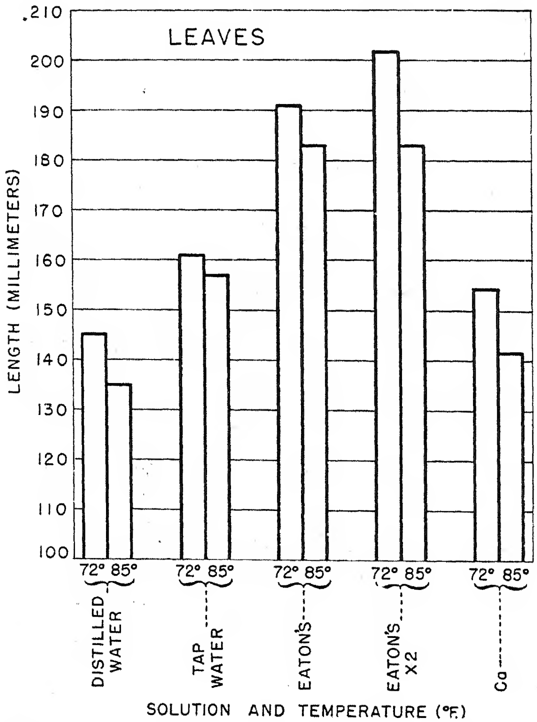

FIGURE 3.—Mean length of leaves of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

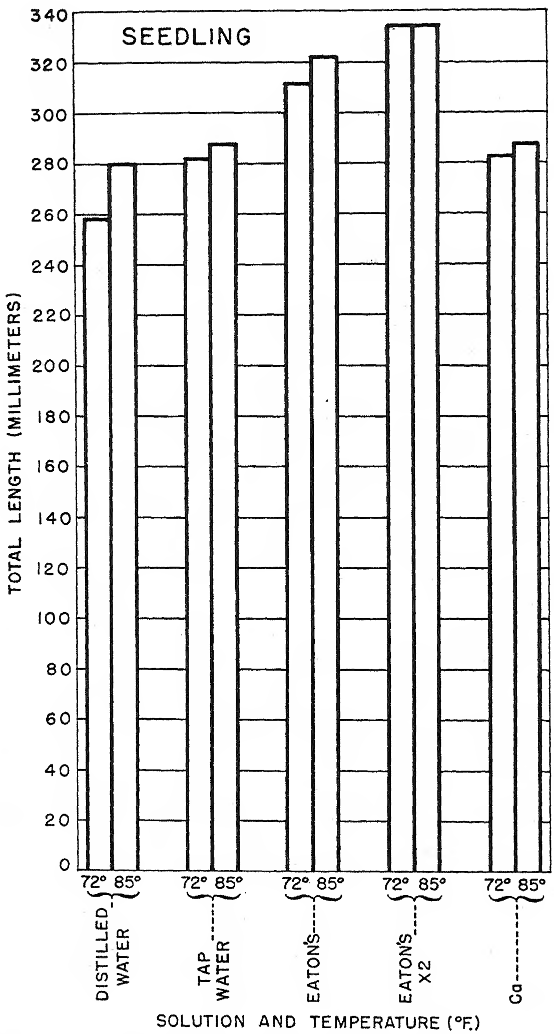

FIGURE 4.—Mean length of entire seedlings of maize grown in five cultures (p. 186) in the dark at 72° and 85° F.

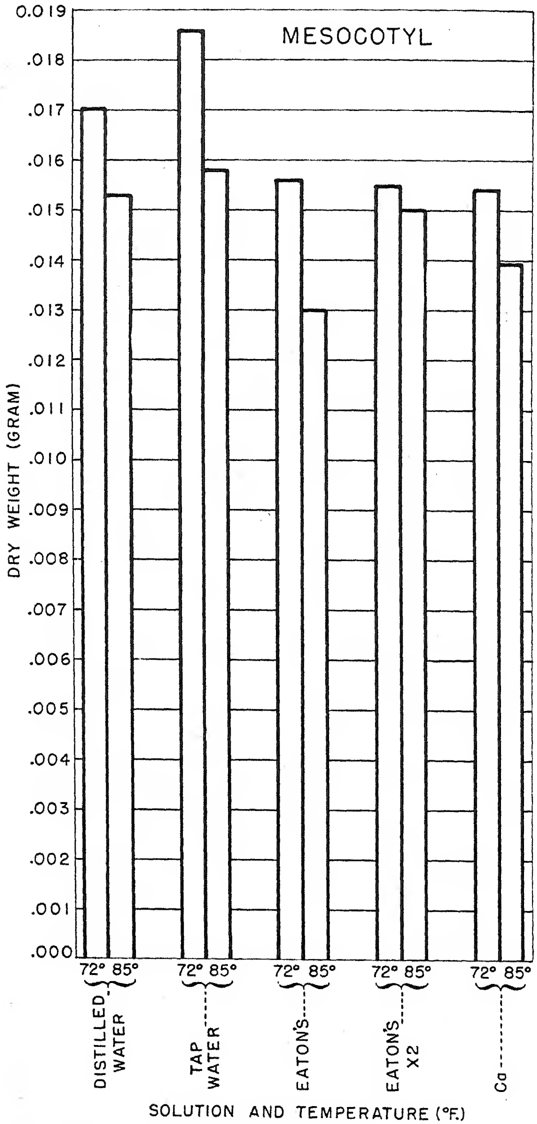

FIGURE 5.—Mean oven-dry weight of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

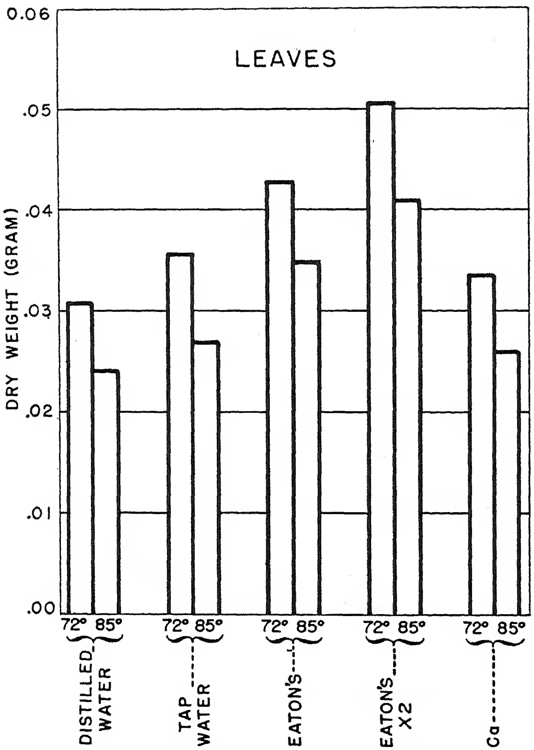

FIGURE 6.—Mean oven-dry weight of leaves of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

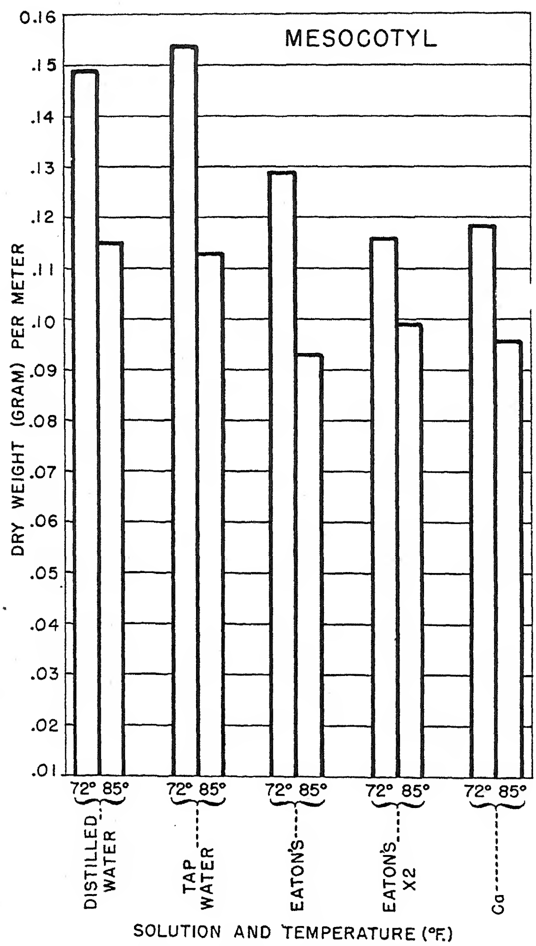

FIGURE 7.—Mean oven-dry weight per meter of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

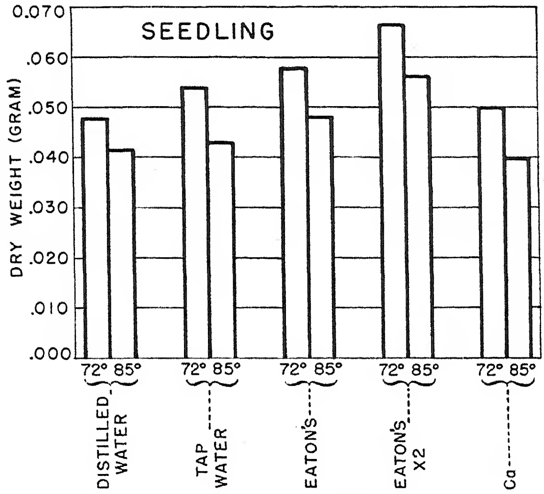

FIGURE 8.—Mean oven-dry weight of entire seedlings of maize grown in five cultures (p. 186) in the dark at 72° and 85° F.

TEMPERATURE EFFECTS

With all the cultures the length of the mesocotyl at the higher temperature was significantly greater than the length at the lower temperature for the corresponding culture. There was no temperature effect on length of coleoptile or tops or on total length.

There was a temperature effect on weight of mesocotyl such that the mesocotyls at the lower temperature were the heaviest, and this was true also for the weight of tops, which was significantly greater at the lower temperature in all the cultures except the distilled water.

In all cultures the weight per meter of mesocotyl was greater at the lowest temperature, and in every case the entire plants weighed more at the lowest temperature.

The entire plant at this stage weighed approximately 15 percent of the dry weight of the seed when planted.

SOLUTION EFFECTS

Taking the plants in distilled water as the base, the following facts are demonstrable:

(1) At the lower temperature the cultures in the concentrated solution and in the calcium solution have significantly longer mesocotyls.

(2) At the higher temperature there are no significant differences in mesocotyl length.

(3) At both temperatures the coleoptiles are significantly longer in the two complete solutions.

(4) At both temperatures the tops are significantly longer in the two complete solutions.

(5) At both temperatures the entire seedlings are significantly taller in the two complete solutions.

(6) At neither temperature are the weights of mesocotyls affected by the solutions.

(7) At both temperatures the weights of tops are significantly greater in the two complete solutions.

(8) At the lower temperature the weight per meter of mesocotyl is significantly less in the two complete solutions and in the calcium solution.

(9) At the lower temperature the seedlings grown in tap water and in the two complete solutions are significantly heavier.

(10) At the higher temperature only the seedlings grown in the concentrated solution are significantly heavier.

From the above results, it is apparent that the two complete solutions have a profound effect on the elongation of the maize seedling growing without light and on the transfer of stored material from the seed. This effect is greater at temperatures below the optimum. On the other hand, it is equally apparent that except for the size of mesocotyl the active element in the complete solution is not calcium. Calcium, however, appears to be the activating element in mesocotyl lengths, as indicated by the total length and the reduced weight per meter. This suggests that the role of calcium, insofar as it affects this organ, is one of stimulation of elongation rather than the speeding up of the translocation of stored material from the endosperm.

It may be concluded that, although calcium in the solution will insure the complete elongation of the mesocotyl, the other salts present in the complete solutions are needed for the greatest development of the plant parts above the mesocotyl.

From the reactions at the two temperatures it may be concluded that the function of the salts in the solution is to speed the transfer of stored material to the leaves.

EFFECT OF DISTILLED WATER AND COMPLETE CULTURE SOLUTION AT CONSTANT TEMPERATURE

Although it has been shown by the three previous experiments that seedlings grown in culture solutions in the dark are larger than those grown in distilled water, the possibility remains that the solutions do not increase the ultimate size of the seedlings but act merely as accelerating agents, speeding the transformation of the stored starch and the translocation of the resulting sugars from the endosperm to the seedling. It was not practicable to prolong the experiments until all seedling growth had ceased, because of the break-down of tissue from decay; therefore, in the experiments thus far conducted, if the solutions increased the rate of translocation, this would be reflected as an increase in the size of the seedling.

As a preliminary investigation of the effect of solution on the speed of seedling growth, the following experiment was started June 14 in a chamber operating at a constant temperature of 72° F. In this experiment 24 soil cans were used, each containing 575 gm. of coarse, oven-dry sand, the particles of which passed a 1/12-inch sieve and were caught on a 1/18-inch mesh.

Each can was planted with 20 seeds of Funk 1939 stock, but the weights of this seed were not obtained. After planting, 12 cans were wet with 110 cc. of Eaton's solution at twice the published concentration, and 12 were wet with 110 cc. of distilled water. The cans were then covered with tin tubes and placed in the 72° F. chamber. Beginning on June 19, 5 days after planting, 2 cans from each solution were removed at random daily with the exception of June 23 (Sunday), giving 6 samplings. Measurements were recorded for mesocotyls, coleoptiles, and tops. These parts, as products of single cans, together with the roots, were oven-dried separately and weighed. The data are given in table 6, and shown graphically in figures 9 to 16. The analyses of variance are given in table 7.

TABLE 6.—*Mean lengths and weights of seedling parts grown in the dark at a constant temperature of 72° F. in the media indicated*

Culture	Days from planting	Length			Oven-dry weight						
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Seed residue	Total recovered dry matter	Weight of mesocotyl per meter	Dry matter translocated
		Millimeters	*Millimeters*	*Millimeters*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Distilled H_2O	5	58.65	15.47	[1] 15.47	0.0128	0.0060	0.0137	0.2337	0.2662	0.2176	0.0325
Solution		63.72	18.10	18.10	.0136	.0072	.0142	.2477	.2828	.2139	.0350
Distilled H_2O	6	99.30	27.06	27.06	.0219	.0054	.0183	.2236	.2745	.2212	.0456
Solution		108.05	30.00	30.00	.0211	.0072	.0169	.2102	.2606	.2009	.0452
Distilled H_2O	7	122.42	41.94	43.50	.0256	.0161	.0231	.1973	.2622	.2095	.0648
Solution		138.95	60.02	72.01	.0257	.0266	.0259	.1742	.2525	.1855	.0782
Distilled H_2O	8	151.77	51.05	59.35	.0285	.0192	.0263	.1807	.2549	.1882	.0740
Solution		157.55	72.32	117.29	.0254	.0367	.0260	.1553	.2435	.1614	.0881
Distilled H_2O	10	171.97	58.72	82.02	.0291	.0235	.0273	.1528	.2327	.1688	.0799
Solution		172.71	75.19	172.46	.0246	.0528	.0226	.1286	.2287	.1431	.1000
Distilled H_2O	11	172.65	54.43	82.18	.0259	.0213	.0236	.1529	.2238	.1503	.0708
Solution		169.16	72.54	196.83	.0222	.0614	.0249	.1035	.2121	.1313	.1085
Difference required for significance ($P=0.01$)		22.70	9.90	29.70	.0052	.0450	.0030	.0300	.0273	.0300	

[1] For the first 2 samples, tops and coleoptiles are identical as no leaves were exserted.

TABLE 7.—*Analyses of variance based on means of 24 cans of 20 seedlings for the seedling parts shown*

Source of variation	Degrees of freedom	Mean square								
		Length			Weight					
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Seed residue	Total recovered dry matter	Weight per meter of mesocotyl
Total	23	1,698.27	440.09	3,404.33	0.000027	0.00438	0.000023	0.00190	0.000491	0.000981
Culture	1	185.76	[1] 1,053.37	[1] 14,710.91	[1] .000030	[1] .00168	.000003	[1] .00246	.000201	[1] .002380
Time	5	[1] 7,578.32	[1] 1,718.90	[1] 10,321.79	[1] .000110	[2] .00091	[1] .000096	[1] .00757	[1] .001891	[1] .003719
Interaction	5	47.22	[1] 68.42	[1] 2,159.60	.000002	.00024	[1] .000006	[2] .00042	.000129	.000072
Error	12	62.23	11.02	98.47	.000003	.00022	.000001	.00010	.000083	.000102

[1] $P<0.01$.
[2] $P<0.05$.

The effect of the culture solution was greatest on the tops. At the termination of the experiment, the dry matter in the tops was at its highest point, and this is true also for the length of tops, while the plants in distilled water had attained their limit.

There was no demonstrable effect of the culture solution on weight of roots.

In conformity with the effect of solutions on seedling parts, the seed residues show that the culture solution accelerated the translocation of material from the seed and removed a greater total amount.

In weight per meter of mesocotyl the culture solution produced plants with less dry matter for unit of length in this organ. The ratio of dry matter to unit length declined with length, and therefore with time. Apparently increase in length is at least partly at the expense of diameter.

The measurements show that the experiment continued until the mesocotyls had begun to shrink in length and lose in weight. It is apparent that the culture solution used accelerated the elongation of the mesocotyl but did not increase its final length, and the numbers were too small to establish the significance of this interaction. On the other hand, the solution not only hastened the elongation of coleoptiles and tops, but it also resulted in an increase in their final length. Evidently the seedlings in the distilled water had reached their final size before the conclusion of the experiment, which would indicate that the salt solution facilitated the translocation of the stored products from the seed. It is possible that in distilled water the loss of solutes from the seed to the solution becomes a limiting factor on further translocation. However, other experiments show that the loss of dry matter to the solution is only about 5 percent more in the distilled water than in the solution.

At every sampling date after the first, the dry matter in the mesocotyls of seedlings grown in distilled water exceeded that of seedlings grown in the solution. By the third sampling date, the mesocotyls of seedlings grown in the solution had reached their maximum dry-matter content and declined from then on, probably because of the drain made by the rapidly growing tops.

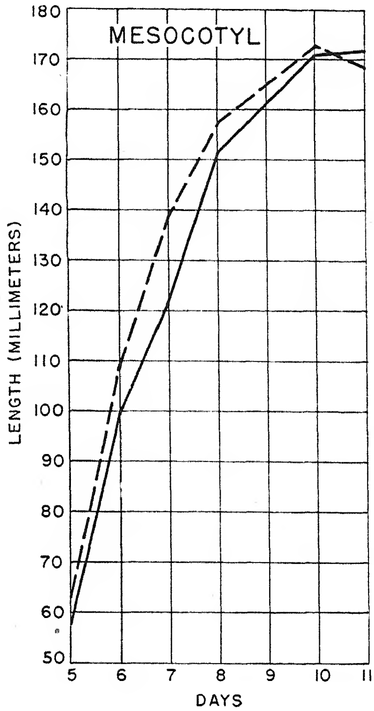

FIGURE 9.—Increase in length of mesocotyls of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

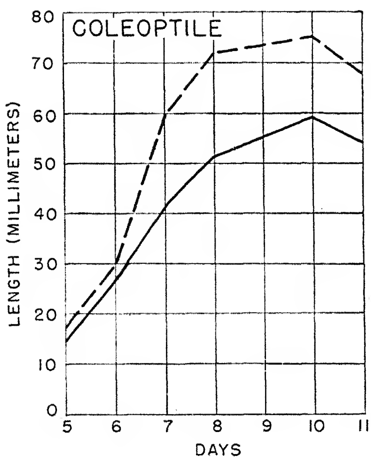

FIGURE 10.—Increase in length of coleoptiles of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

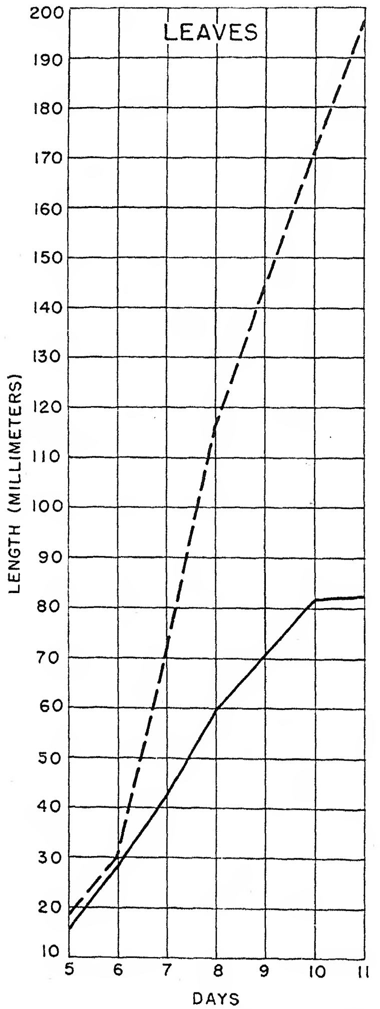

FIGURE 11.—Increase in length of leaves of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

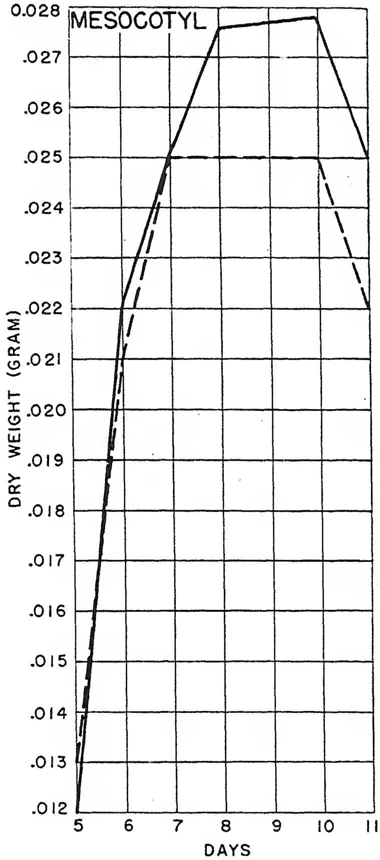

FIGURE 12.—Increase in weight of mesocotyls of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

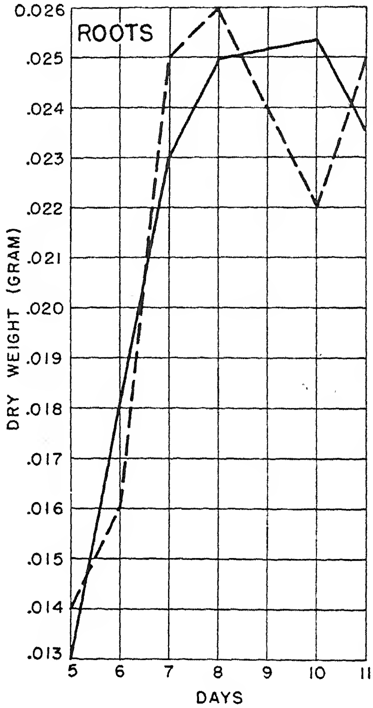

FIGURE 13.—Increase in weight of roots of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

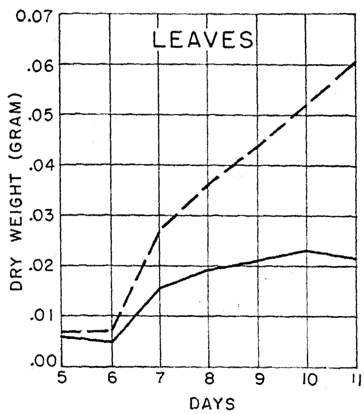

FIGURE 14.—Increase in weight of leaves of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

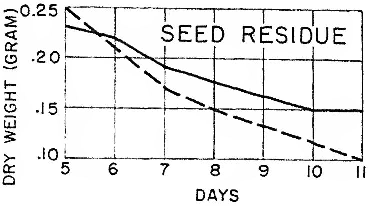

FIGURE 15.—Decrease in weight of seed residue of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

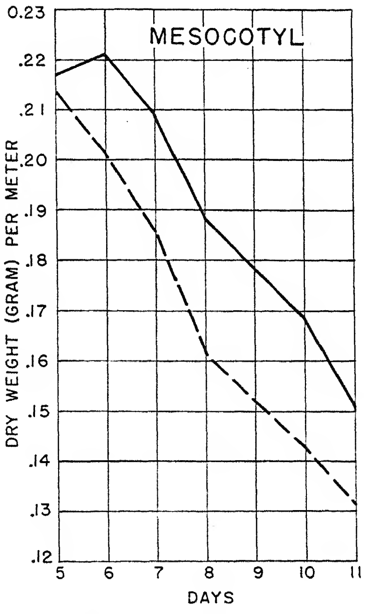

FIGURE 16.—Decrease in weight per meter of mesocotyl of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

EFFECT OF DISTILLED WATER AND TWO SALT SOLUTIONS AT TWO CONSTANT TEMPERATURES

In view of the conclusions drawn from the preceding experiment, it was desirable to repeat the test on a larger scale. Three cultures were used, namely, distilled water, the complete solution given on page 184 at double concentration, and the calcium solution given on page 185 at double the concentration. The two chambers used in the experiment described on page 186 were used, but the temperatures were 69° and 90° F. Thermograph records kept during the experiment show these temperatures to have been maintained with a high degree of uniformity.

The seedlings were grown in tin soil cans, 10 weighed seeds of Funk Yellow Dent corn in each can. The cans contained 550 gm. of oven-dry crushed coarse quartz moistened with 110 cc. of solution. The seeds were random samples from a large mixed lot weighed in 10 seed groups and assigned at random to the cans. Analysis of variance showed that the random arrangement had been achieved. Thirty cans of each solution were planted at each of the two temperatures. Within each temperature the cans were randomly arranged. The seeds were planted January 8, 1941, and 5 days after planting 5 cans were sampled from each solution at each temperature every 24 hours for 6 days thereafter. The seedling parts of the individual seedlings were measured and washed free of quartz particles, after which the products of each can were dried at 100° C. and weighed. The weights were not made on an individual-plant basis but on the product of an entire can of 10 seedlings.

The results are shown in table 8, and the apportioned variances are given in table 9. The measurements show that 90° F. is too high a temperature for the sampling system followed. For comparable, useful thermal units, the growth thermometer might be thought of as covering that section of the Fahrenheit scale lying between 50° and 120°. For equal seedling sizes at temperatures of 69° and 90°, sampling of the 90° cultures should be at intervals one-half the length of those at 69°; for example, where the 69° culture was sampled 5 days after planting, sampling of the 90° culture should have started at 2½ days.

TABLE 8.—*Mean length, weight, and percentage of seed and seedling parts of plants grown in the dark for various periods, at different temperatures, and in 3 culture solutions* [1]

Seedling part	5 days from planting						6 days from planting						7 days from planting					
	90° F.			69° F.			90° F.			69° F.			90° F.			69° F.		
	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.
Length mesocotyl ..... mm	89.04	147.22	156.67	50.16	64.61	63.37	82.50	149.72	159.26	79.88	107.48	116.38	107.00	156.34	167.32	91.89	142.98	152.37
Length coleoptile ..... mm	43.27	67.58	56.02	15.63	16.53	13.90	47.74	64.76	63.44	26.31	34.60	26.60	53.68	66.48	65.06	33.87	47.00	37.35
Length tops ..... mm	43.27	101.40	65.98	15.63	16.53	13.90	53.04	138.54	95.30	24.06	34.60	26.60	85.44	198.98	117.66	29.69	51.28	36.92
Weight mesocotyl ..... gm	.0181	.0248	.0290	.0125	.0141	.0144	.0180	.0227	.0285	.0200	.0227	.0246	.0183	.0194	.0278	.0224	.0281	.0309
Weight tops ..... gm	.0161	.0402	.0273	.0058	.0069	.0064	.0183	.0494	.0357	.0094	.0137	.0105	.0228	.0626	.0379	.0115	.0194	.0140
Weight roots ..... gm	.0284	.0271	.0223	.0137	.0128	.0113	.0288	.0313	.0264	.0183	.0171	.0154	.0253	.0275	.0256	.0210	.0202	.0174
Weight per meter of mesocotyl ..... gm	.2034	.1682	.1850	.2492	.2210	.2268	.2204	.1519	.1790	.2520	.2108	.2114	.1714	.1249	.1680	.2441	.1962	.2072
Weight seed planted ..... gm	.3063	.2962	.3080	.3030	.3005	.3014	.3023	.3009	.3022	.3054	.3009	.3002	.3048	.2968	.2985	.3069	.2919	.3004
Weight seed residue ..... gm	.1511	.1148	.1426	.2407	.2446	.2421	.1322	.0966	.1164	.2136	.2092	.2154	.1144	.0665	.0870	.1903	.1727	.1869
Weight seed lost ..... gm	.0926	.0894	.0869	.0303	.0221	.0272	.1049	.1010	.0952	.0441	.0383	.0342	.1240	.1208	.1202	.0615	.0515	.0512
Weight seed translocated ..... gm	.0626	.0920	.0785	.0320	.0338	.0321	.0652	.1033	.0906	.0477	.0534	.0506	.0664	.1095	.0913	.0551	.0677	.0623
Weight dry matter recovered ..... gm	.2137	.2068	.2311	.2727	.2784	.2742	.1974	.1999	.2070	.2613	.2626	.2660	.1808	.1760	.1783	.2454	.2404	.2492
Recovered dry matter translocated ..... pct	29.29	37.96	33.97	11.73	12.14	11.71	33.03	51.68	43.77	18.25	20.34	19.02	36.73	62.22	51.21	22.45	39.20	25.00
Recovered dry matter in mesocotyl ..... pct	8.47	11.99	12.55	4.58	5.06	5.25	9.12	11.35	13.77	7.65	9.37	9.25	10.12	11.02	15.59	9.13	11.69	12.40
Recovered dry matter in tops ..... pct	7.53	19.44	11.82	2.13	2.48	2.33	9.27	24.71	17.25	3.60	5.22	3.95	12.61	35.57	21.26	4.69	8.07	5.62
Recovered dry matter in roots ..... pct	13.29	13.10	9.65	5.02	4.60	4.12	14.59	15.66	12.75	7.00	6.51	5.79	13.99	15.62	14.36	8.56	8.40	6.98
Dry matter lost ..... pct	30.23	30.18	28.21	10.00	7.35	9.02	34.70	33.57	31.50	14.44	12.73	11.39	40.68	40.70	40.27	20.04	17.64	17.04
Dry matter in seed residue ..... pct	49.33	38.76	46.30	79.44	81.40	80.33	43.73	32.10	38.52	66.44	69.52	71.75	37.53	22.41	29.15	62.01	59.16	62.22
Dry matter translocated ..... pct	20.43	31.06	25.49	10.56	11.25	10.65	21.56	34.33	29.98	15.62	17.75	16.86	21.78	36.89	30.59	17.95	23.19	20.74

See footnote at end of table.

TABLE 8.—*Mean length, weight, and percentage of seed and seedling parts of plants grown in the dark for various periods, at different temperatures, and in 3 culture solutions* [1]—Continued

Seedling part	8 days from planting						9 days from planting						10 days from planting					
	90° F.			69° F.			90° F.			69° F.			90° F.			69° F.		
	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca. sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.
Length mesocotyl...mm	82.78	158.33	162.08	101.48	151.60	175.94	107.59	169.76	163.47	110.22	158.18	184.56	104.43	164.31	174.65	110.84	164.80	181.72
Length coleoptile...mm	45.40	65.71	64.20	41.36	63.60	46.64	50.04	64.38	62.63	50.00	66.16	55.60	51.27	67.08	64.59	50.04	70.54	58.18
Length tops...mm	74.66	230.29	147.65	44.80	83.96	49.52	83.98	260.94	159.39	59.84	130.43	66.48	146.88	281.71	211.86	77.61	188.80	94.54
Weight mesocotyl...gm	.0163	.0182	.0245	.0255	.0294	.0351	.0159	.0183	.0225	.0243	.0274	.0334	.0158	.0159	.0213	.0251	.0249	.0323
Weight tops...gm	.0202	.0610	.0451	.0165	.0300	.0179	.0180	.0324	.0389	.0180	.0414	.0215	.0270	.0570	.0407	.0222	.0585	.0260
Weight roots...gm	.0319	.0252	.0267	.0234	.0229	.0182	.0246	.0245	.0234	.0232	.0244	.0201	.0258	.0238	.0222	.0283	.0309	.0231
Weight per meter of mesocotyl...gm	.1989	.1178	.1512	.2521	.1968	.1999	.1473	.1085	.1386	.2277	.1739	.1821	.1532	0974	.1219	.2279	.1517	.1784
Weight seed planted gm	.2927	.2986	.3096	.3067	.2961	.2961	.3035	.2921	.3088	.3067	.3030	.2976	.3120	.3064	.3122	.3033	.3032	.3054
Weight seed residue...gm	.0870	.0561	.0745	.1785	.1462	.1704	.0887	.0512	.0729	.1621	.1246	.1535	.0706	.0465	.0627	.1396	.0898	.1342
Weight seed lost...gm	.1372	.1378	.1388	.0629	.0676	.0545	.1563	.1357	.1511	.0790	.0852	.0692	.1728	.1631	.1654	.0881	.0991	.0897
Weight seed translocated gm	.0685	.1047	.0963	.0653	.0823	.0712	.0585	.1052	.0848	.0656	.0932	.0749	.0686	.0968	.0841	.0756	.1143	.0815
Weight dry matter recovered...gm	.1555	.1608	.1708	.2438	.2285	.2416	.1472	.1564	.1577	.2277	.2178	.2283	.1392	.1433	.1468	.2152	.2041	.2157
Recovered dry matter translocated...pct	44.05	65.11	56.38	26.78	36.02	29.47	39.74	67.26	53.77	28.81	42.79	32.81	49.28	67.55	57.29	35.13	56.00	37.78
Recovered dry matter in mesocotyl...pct	10.48	11.32	14.34	10.54	12.87	14.53	10.80	11.70	14.27	10.67	12.58	14.63	11.35	11.10	14.51	11.66	12.20	14.97
Recovered dry matter in tops...pct	12.99	37.94	26.41	6.77	13.13	7.41	12.23	39.90	24.67	7.91	19.01	9.42	19.40	39.78	27.72	10.32	28.66	12.05
Recovered dry matter in roots...pct	20.51	15.67	15.63	9.60	10.02	7.53	16.71	15.66	14.84	10.19	11.20	8.80	18.53	16.61	15.12	13.15	15.14	10.71
Dry matter lost...pct	46.87	46.15	44.83	20.51	22.83	18.41	51.50	46.46	48.93	25.76	28.12	23.25	55.38	53.23	52.98	29.05	32.68	2.937
Dry matter in seed residue...pct	29.72	18.79	24.06	58.20	49.38	57.55	29.23	17.53	23.61	52.85	41.12	51.57	22.63	15.18	20.08	46.03	20.62	43.94
Dry matter translocated pct	23.40	35.06	31.10	21.29	27.79	24.05	19.28	36.02	27.46	21.39	30.76	25.17	21.99	31.59	26.94	24.93	37.70	26.69

[1] Dist. H_2O=distilled water; E. sol.=Eaton's solution (p. 184) doubled in concentration; Ca sol.=solution of calcium salts (p. 185) doubled in concentration.

TABLE 9.—*Analyses of variance based on means of 10 seedling groups for the seedling parts and the treatments shown*

Source of variation	Degrees of freedom	Mean square									
		Length			Weight						
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Per meter of mesocotyl	Seed residue	Dry matter lost	Dry matter translocated
Total	179	1,593.81	272.48	5,313.54	0.000037	0.00031	0.000029	0.00193	0.00628	0.001963	0.00062
Culture	2	[1] 65,325.48	[1] 3,536.15	102,541.59	[1].000910	[1].09933	[1].000205	[1].04236	[2].01292	[1].000566	[1].01103
Temperature	1	[1] 12,036.05	[1] 13,237.94	291,958.77	[1].000716	[1].01523	[1].001647	[1].13890	[1].33547	[1].213880	[1].01883
Time	5	[1] 13,700.85	[1] 2,624.41	[1] 6,077.71	[1].000142	[1].00179	[1].000145	[1].01388	[1].04182	[1].021292	[1].00386
Interactions:											
Culture-temperature	2	[1] 1,677.01	[1] 429.57	[1] 23,450.53	[1].000014	[1].00171	.000010	[1].00164	.00058	[1].000294	[1].00175
Culture-time	10	[1] 435.95	40.17	4,055.86	[2].000010	[1].00021	[2].000010	[1].00064	.00033	.000006	.00019
Temperature-time	5	[1] 11,791.93	[1] 1,923.42	2,013.23	[1].000496	[1].00043	[1].000298	[2].00065	.00247	[1].000280	[1].00287
Solution-temperature-time	10	[1] 535.17	[1] 77.68	1,058.96	[1].000019	[1].00015	[1].000017	.00034	.00055	[1].001503	.00017
Error	144	176.11	24.53	292.16	.000004	.00002	.000004	.00025	.00369	.000009	.00021

[1] $P<0.01$.
[2] $P<0.05$.

The results of this experiment did not confirm the data, obtained from the preceding experiment, on the effect of the complete solution on length of mesocotyl. A comparison of figures 9 and 17 shows that, while the growth curves in the two experiments are very similar in rate and final size for the plants grown in the complete culture solution, they are astonishingly different for the plants grown in the distilled water. In the experiment illustrated in figure 9, the length of mesocotyl in distilled water is equal to that in the complete solution and both are very long.

For the seedling parts above ground, the analyses of variance (table 9) show significant interactions of solutions with temperature. The nature of these interactions is shown in figures 17 to 21. With the exception of measures of mesocotyl development, at 69° F. the plants in the calcium solution were more nearly like those in distilled water, and at 90° these plants were intermediate between those in distilled water and those in the complete solution. The root development shows no interaction of solution with temperature, though there is a significant triple interaction of solution, temperature, and time.

Length of Mesocotyl

It is evident that at the first sampling date the mesocotyls in all three solutions in the 90° F. culture were approaching their maximum length. Only the upper reaches of the curves are available (fig. 17, *A*). It is clear, however, that the solution of calcium salts produces the longest mesocotyls, although there is not much difference in this respect between the two nutrient solutions. In the 69° culture both nutrient solutions clearly accelerated the elongation of the mesocotyls and carried them to a greater final length than did distilled water (fig. 17, *B*). This is not in accordance with the results first obtained (p. 197 and fig. 9). The calcium solution was more effective in stimulating the elongation of the mesocotyl than was the complete solution, thus confirming the results of the previous experiments.

Length of Coleoptile

Both solutions were more effective than distilled water in producing long coleoptiles at 90° F. The complete solution produced the longest coleoptiles, but the two solutions were very similar in this respect (fig. 18, *A*). At 69° the differences in coleoptile length between the three solutions were not so great (fig. 18, *B*). The coleoptiles in the calcium solution and in distilled water were much more nearly the same size at 69° than at 90°. As at 90°, the complete solution produced the longest coleoptiles at 69°.

Length of Tops

At 90° F. the true leaves were very much longer in the complete solution and grew faster than in the other solutions (fig. 19, *A*). The calcium solution produced leaves midway in length between those in the complete solution and those in distilled water. At 69° the lengths of leaves in the calcium solution and in the distilled water were very nearly alike, whereas the complete solution produced long, rapidly elongating leaves (fig. 19, *B*).

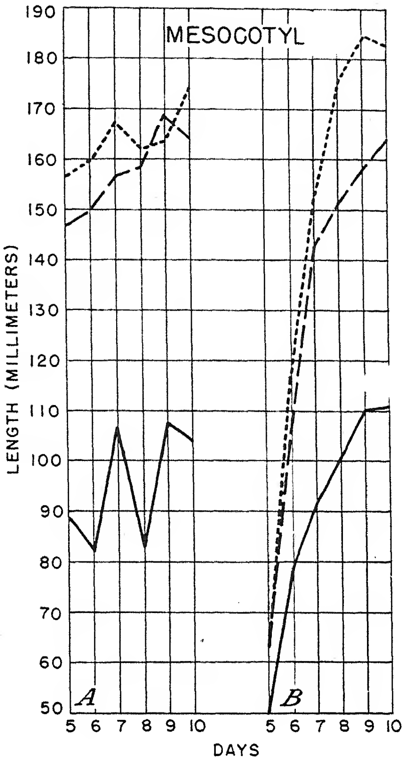

FIGURE 17.—Increase in length of mesocotyls of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

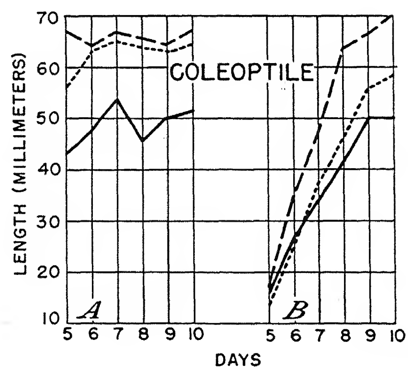

FIGURE 18.—Increase in length of coleoptiles of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

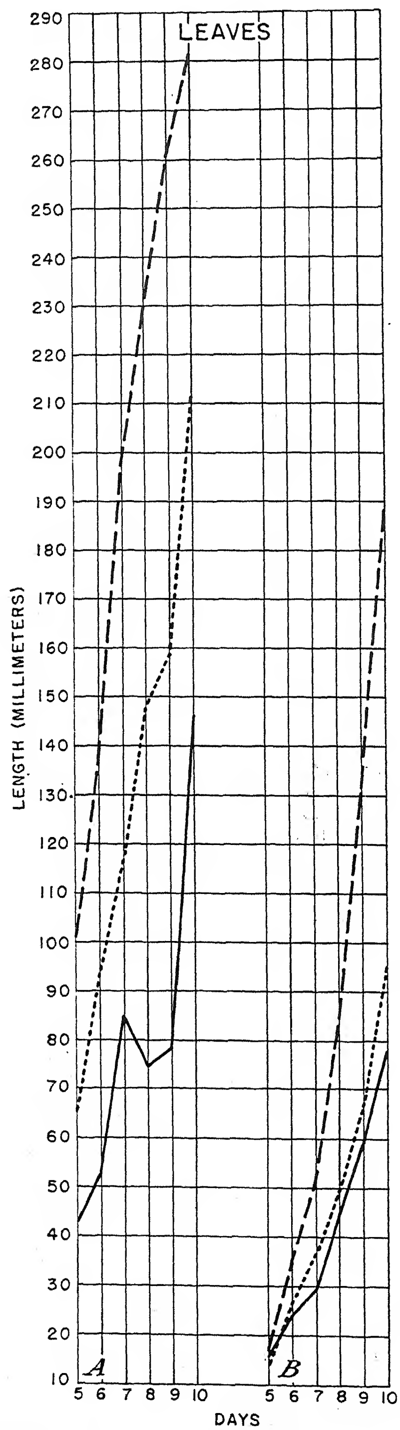

FIGURE 19.—Increase in length of leaves of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

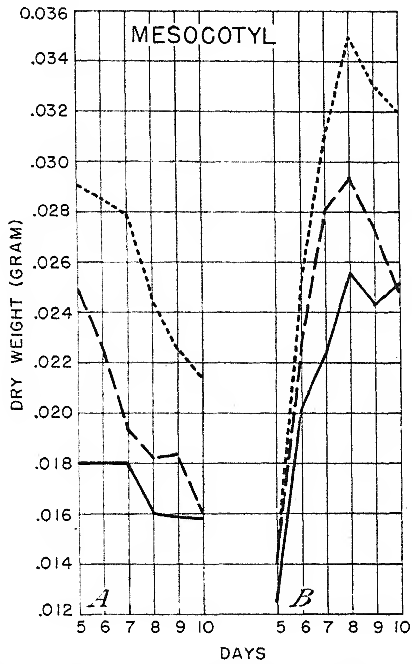

FIGURE 20.—Increase in weight of mesocotyls of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

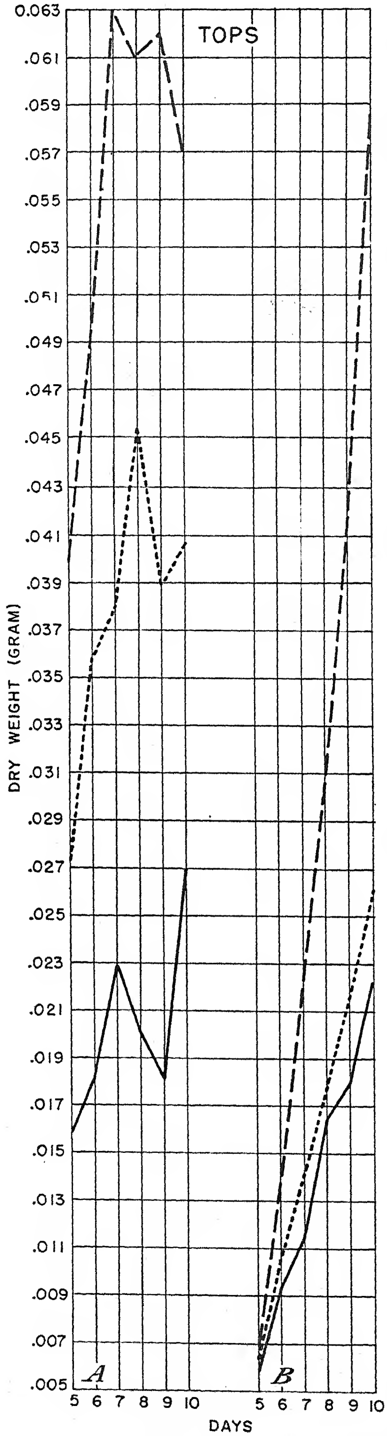

FIGURE 21.—Increase in weight of tops of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

Weight of Mesocotyl

The relatively late sampling period for the 90° F. culture is shown graphically in figure 20, *A*, where the dry weights of mesocotyls are greatest on the first sampling day and decline each day thereafter. The mesocotyls were heaviest in the calcium solution, which would be expected to follow from the greater length of this organ in this solution. The loss in dry matter is probably the result of the drain made by the rapidly elongating leaves, which doubtless pull sustenance from the mesocotyl as well as from the seed. In the 69° culture the dry weights of mesocotyls increase with time in all three solutions, until at 9 days after planting all three start to decline (fig. 20, *B*). The leaves at this temperature are exserted from the coleoptile 7 days after planting, but apparently the seed is able to provide food for both leaves and mesocotyls as long as these organs are located close to the source of supply. At 8 days the leaf node is 16 to 18 cm. above the seed in the culture solutions and the mesocotyl begins to lose dry matter to the leaves. At 69°, as at 90°, the dry matter in the mesocotyls is greatest in the calcium solution.

Weight of Tops

The weight of the tops at 90° F. reached its maximum in the complete solution 7 days after planting and in the calcium solution 8 days after planting (fig. 21, *A*). In distilled water the weights were irregular, but the heaviest plants were obtained on the last sampling date, 10 days after planting. The weight of dry matter represented by the leaves was greatest in the complete solution, least in distilled water, and almost the mean of these two in the calcium solution. At 69° the greatest weights in all three solutions were obtained 10 days after planting (fig. 21, *B*). Here distilled water and the calcium solution were not greatly different in the dry weight of leaves produced. The complete solution was outstanding, with leaves twice as heavy as those in the calcium solution.

Weight of Roots

The weight of roots in the 90° F. culture was very irregular, but the heaviest roots were found in distilled water and the lightest in the calcium solution (fig. 22, *A*). The weight of roots in complete solution was very similar to that in the distilled water. The weight declined in all three cultures before the final sampling date, indicating a loss of dry matter to the upper parts of the seedling. In the 69° culture the weight of roots increased rapidly with time in all three solutions and was greatest at the close of the experiment (fig. 22, *B*). The calcium solution was distinctly a poor medium for root development in comparison with the complete solution and the distilled water, which were very similar in their effect on roots.

Weight of Mesocotyl per Unit Length

As a means of expressing the weight per unit of length of mesocotyls, the weight per meter was chosen. At both temperatures and in all solutions, the weight per meter declined with time (fig. 23, *A* and *B*). As the mesocotyls increased in length, they decreased in dry weight per meter. The weight per meter was greatest in the distilled water

at both temperatures and for all sampling periods and conversely was lightest in Eaton's solution.

From one point of view it could be assumed that a certain amount of dry matter was apportioned to the mesocotyl, and, as this organ became longer with age or by nutritional stimulation, the amount of dry matter per unit length necessarily became less. However, the calcium salts produced the longest mesocotyls but not the lightest in weight per unit of length, and the actual weight of dry matter in the mesocotyl was greater in the two solutions than in distilled water. The most probable explanation for the loss in unit weight with time and with nutritional stimulation is that both these conditions advance

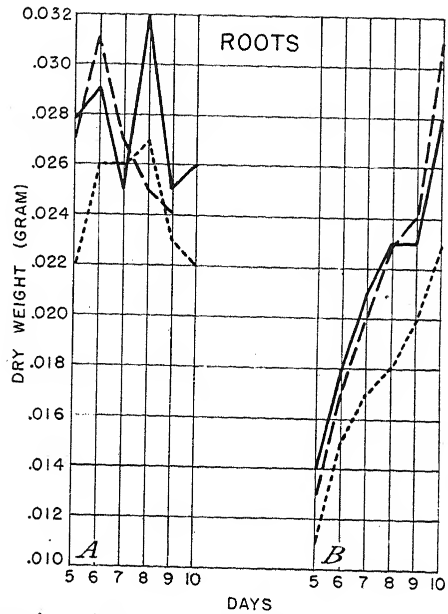

FIGURE 22.—Increase in weight of roots of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

the seedling ontogeny, with the result that the developing leaves begin to constitute a drain on the nutrients in the mesocotyls. Under these conditions weight per unit length would decrease with length and time.

Weight of Seed Residue

The amount of material remaining in the seed, of course, decreased with time. At 90° F. the reserve material remaining in the seed was greatest in distilled water and least in Eaton's solution (fig. 24, *A*). The calcium solution was approximately midway between these two at all sampling periods. The curves show that for this temperature the maximum rate of decline had been reached at the time of the first sampling date, and at 7 days after planting the rate of decline had distinctly slowed.

At 69° F. the solutions stood in the same order as at 90°, but the distilled water and calcium solution were almost identical (fig. 24, *B*). The rate of decline was much greater in all solutions at this temperature than at 90°, but this doubtless is due to the relatively earlier

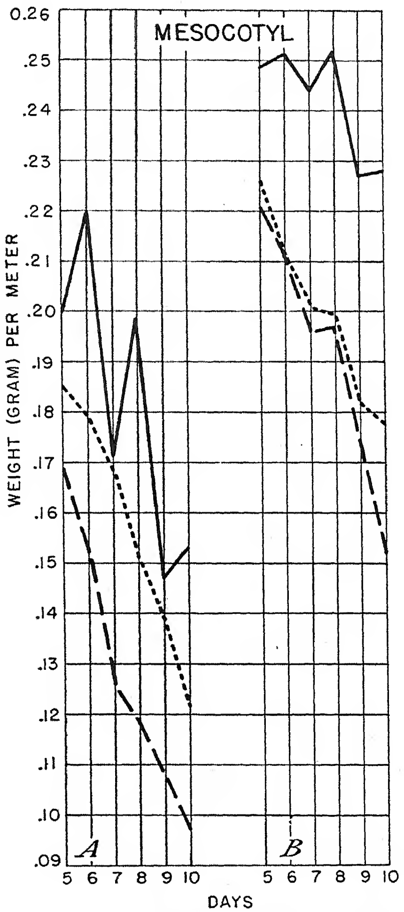

Figure 23.—Decrease in weight per meter of mesocotyl of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

sampling period. At the close of the experiment there was no evidence of a slackening in rate of decline.

Weight of Dry Matter Recovered and of Dry Matter Lost

The weights of dry matter recovered and of dry matter lost are complementary. The amount of dry matter recovered declines rapidly with time, and, conversely, the amount of dry matter lost

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increases rapidly with time (fig. 25, *A*, *B*, and fig. 26, *A*, *B*). In measurements of this nature there are necessarily some unavoidable losses due to handling, despite meticulous care. A certain loss of small hair roots is known to have occurred, and the total weight lost

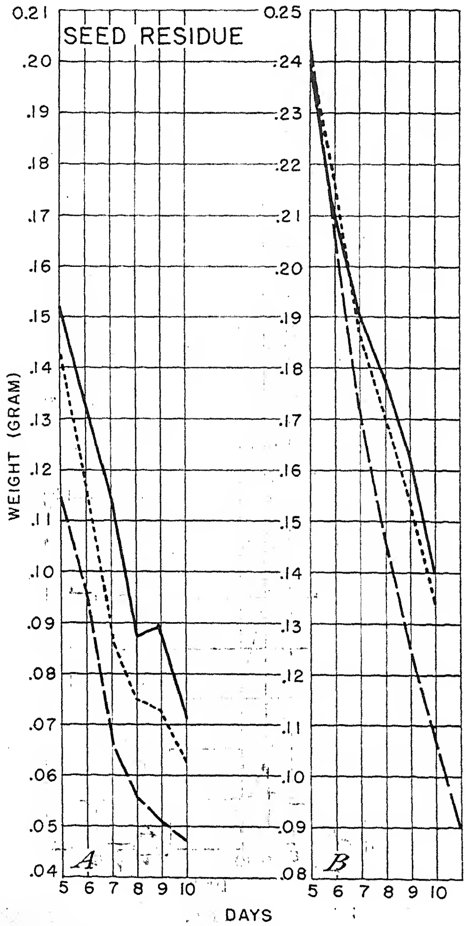

FIGURE 24.—Decrease in weight of seed residue of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

through this factor would increase with the increasing size of the seedlings, because as the plants became larger not only were there more roots to lose but also it was more difficult to separate the seedlings from the quartz. More agitation in water was required to remove the quartz, and consequently there was an increased loss of small roots.

However, only a fraction of the lost dry matter can be accounted for by losses in handling. The principal cause of loss was undoubtedly the presence of micro-organisms; this was reflected in the greater losses at the higher temperatures, as the cultures were not sterile.

A second large factor in the loss of weight must be the energy utilized in the process of translocation with elongation, starch con-

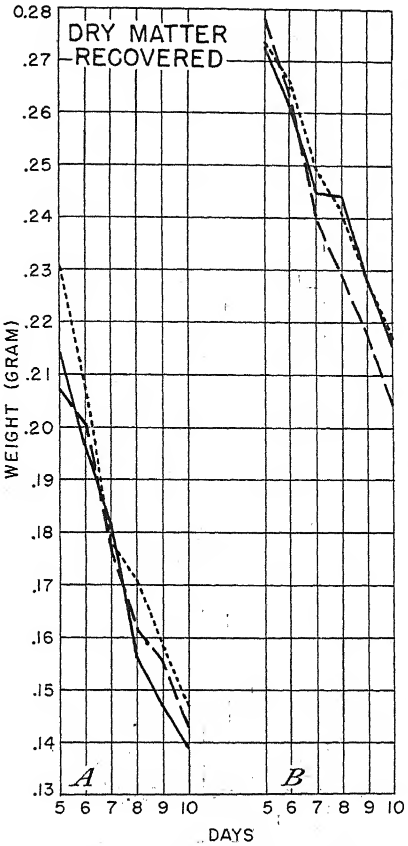

FIGURE 25.—Decrease in weight of total dry matter recovered from maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

version, oxidation, etc. There is a third factor, namely, the loss of solutes to the solutions. Were this an important factor, the distilled-water cultures would show the greatest loss, and to some extent they do, although the differences between the three cultures are not consistent at all sampling dates.

WEIGHT OF DRY MATTER TRANSLOCATED

In both temperature series the greatest quantity of dry matter removed from the seed to the plant occurred in Eaton's solution (fig.

27, *A*). At 90° F. in the complete solution the maximum amount of dry matter had been translocated 7 days after planting, whereas in the calcium solution and in the distilled water the maximum was reached at 8 days. The calcium solution stood almost midway between the distilled water and the complete solution at 90° but not at 69°, where the curves for distilled water and calcium are closely parallel. At 69° in the complete solution the rate of dry matter trans-

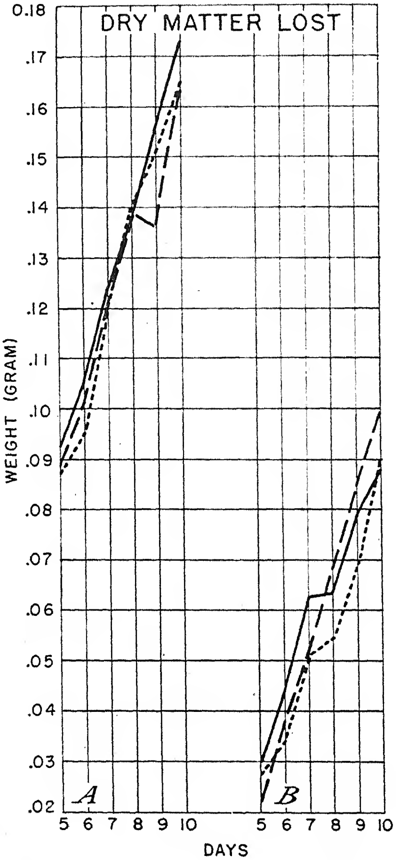

FIGURE 26.—Increase in weight of total dry matter lost of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

located was increasing at the close of the experiment, and the amount of translocated material actually exceeded that for the same solution at 90° (fig. 27, *B*).

PERCENTAGE OF RECOVERED DRY MATTER TRANSLOCATED TO THE SEEDLING

Of the dry matter recovered, the largest percentage translocated to the seedling is found in Eaton's solution at 90° F., where almost 70 percent of the dry matter left at the close of the experiment was found in the seedling (fig. 28, *A*). At this temperature the calcium solution

stood midway between the distilled water and the complete solution. All three groups had passed their maximum rates by the seventh or eighth day from planting.

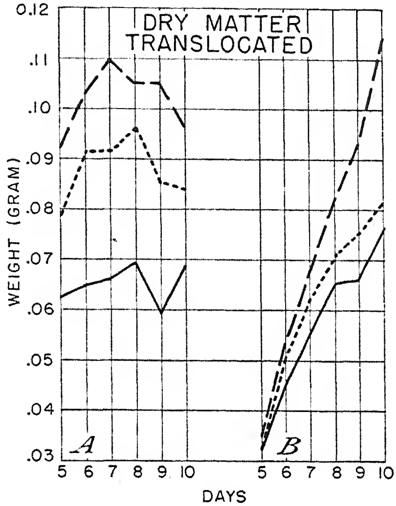

FIGURE 27.—Increase in weight of total dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

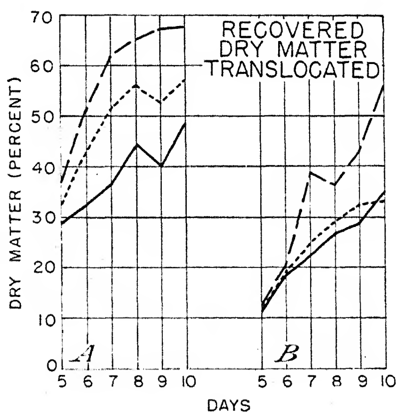

FIGURE 28.—Percentage of recovered dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

At 69° F. the solutions stood in the same order. Even in the complete solution less than 60 percent of the recovered dry matter was found in the seedlings, although the rate was still rising for this solution (fig. 28, *B*). For the distilled water and the calcium solution, which were very closely alike, the rates of transfer had apparently reached

a maximum at the close of the experiment at a level well below that reached at the higher temperature.

PERCENTAGE OF TOTAL RECOVERED DRY MATTER IN TOPS

At the close of the experiment 40 percent of the recovered dry matter was found in the leaves and stems of the seedlings growing in the com-

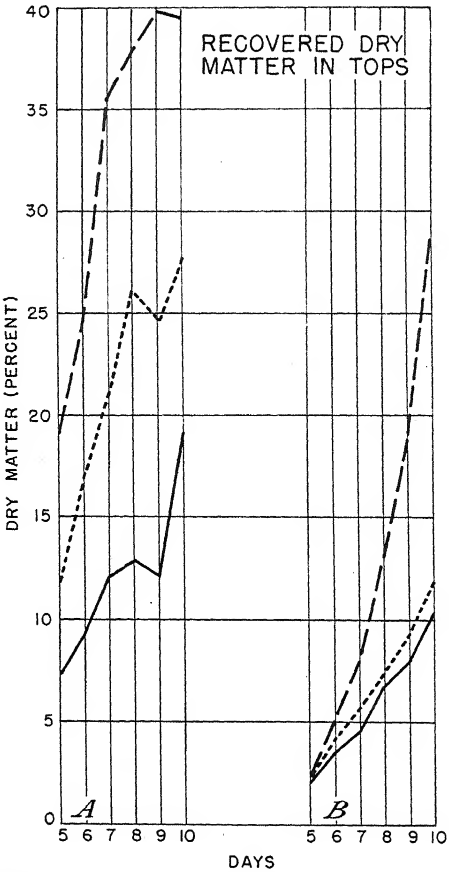

FIGURE 29.—Percentage of recovered dry matter found in tops of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

plete solution at 90° F., and evidently the maximum point had been reached (fig. 29, *A*). The curve for the seedlings growing in the calcium solution falls almost midway between that for distilled water and that for the complete solution.

At 69° F. none of the cultures had reached the maximum point at the close of the experiment. Here the curves for the calcium solution and for the distilled water lie close together and are very similar (fig. 29, *B*).

PERCENTAGE OF TOTAL RECOVERED DRY MATTER IN ROOTS

There was very little difference between the cultures in the percentage of dry matter translocated to the roots, except that at 90° F. the roots in the distilled water clearly exceeded those in the other cultures (fig. 30, *A*). At 69° the percentage of dry matter found in the roots was greatest in the complete solution, though not outstandingly so (fig. 30, *B*). The percentages were uniformly higher at 90°, though at this temperature the maximum point evidently had been

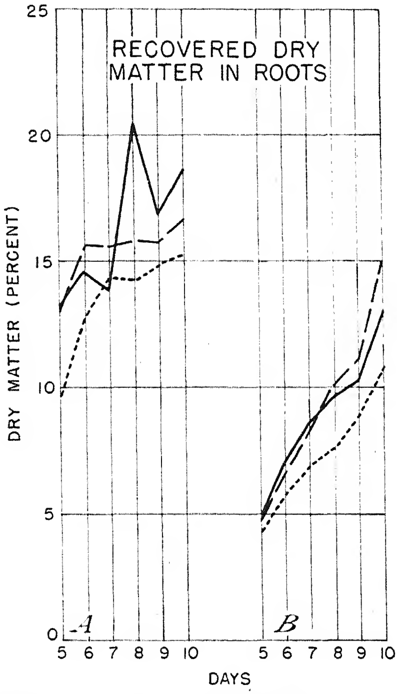

FIGURE 30.—Percentage of recovered dry matter found in roots of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

reached for all three cultures at the time the experiment was terminated. This point had not been reached for any of the cultures at 69°.

PERCENTAGE OF TOTAL RECOVERED DRY MATTER IN MESOCOTYL

At 90° F. the percentage of dry matter in the mesocotyl was greatest for the calcium solution and least for the distilled water (fig. 31, *A*). In general, the percentage declined in the complete solution from the first samples, though the decline was irregular. The lack of agreement in the form of the three curves at 90° may have resulted from the rapid expansion of the leaves in the complete solution in comparison with those in the other two.

At 69° F. the three solutions produced comparable curves until the last two sampling periods, when the percentage declined in the complete solution (fig. 31, *B*).

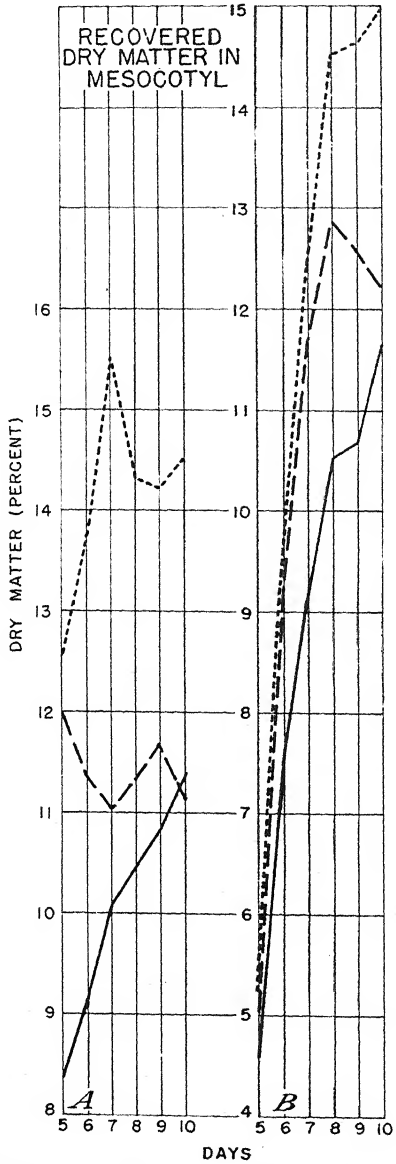

FIGURE 31.—Percentage of recovered dry matter found in mesocotyls of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

PERCENTAGE OF DRY MATTER TRANSLOCATED

The percentage of dry matter translocated from the seeds to seedling parts is shown in figure 32, *A* and *B*. At 90° F. the three solutions stood in the order of Eaton's, calcium, and distilled water, the first-named having the highest percentage. The three curves are very similar in shape, with the maximum percentage at about 8 days after planting. The decline that sets in after this period is the result

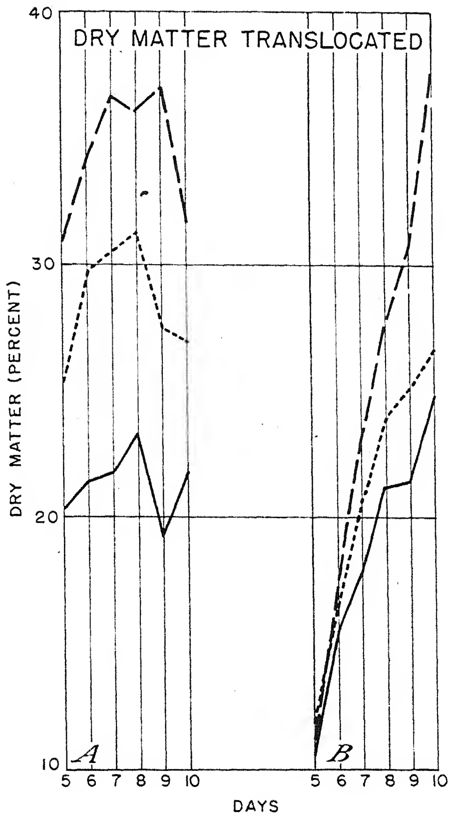

FIGURE 32.—Percentage of original dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

of dry matter lost, probably chiefly to fungi and bacteria. However, this does not furnish a complete explanation, as at 69° the percentage of dry matter translocated increased fairly steadily in all three cultures, and in the complete solution on the last sampling date actually exceeded the maximum found in the 90° group. Further, reference to figure 26, *A* and *B*, shows the two temperature groups to have very similar loss curves. It should be noted that the maximum percentage of dry matter actually moved from the seed to the seedling is less than 40 percent in the most effective culture and less than 25 percent in distilled water.

Percentage of Dry Matter in Seed Residue

Of the dry matter in the seed when planted, that not translocated to the seedling was either left in the seed or lost. Of course, part of the seed material is not usable by the seedling and part of it, though used, remains in the seed, such, for example, as that in the scutellum. At 90° F., at all sampling dates, the seed residues were least in the complete solution and greatest in distilled water, in line with the seedling sizes and percentages of dry matter translocated. It is quite evident from figure 33, *A*, that the decline in weight of seed residue

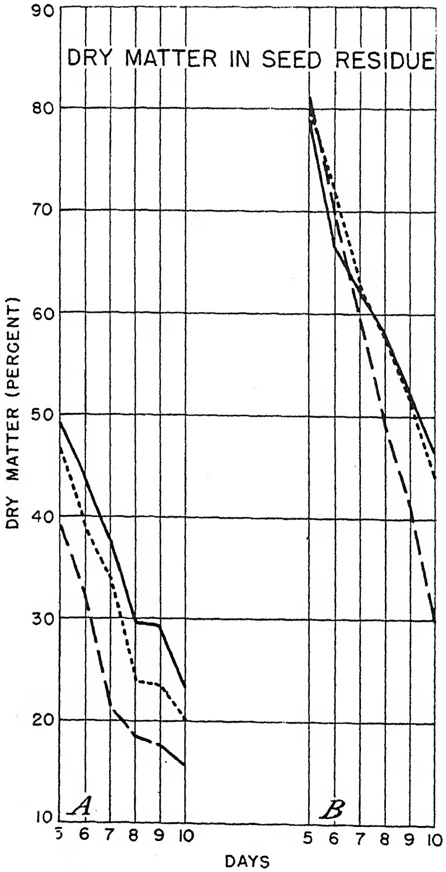

Figure 33.—Percentage of dry matter in seed residue of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

had set in 7 or 8 days after planting.

At 69° F. the percentage of dry matter left in the seed was least in the complete solution. The curves for distilled water and the calcium solution are almost identical (fig. 33, *B*). The rate of loss had not slackened in any of the three cultures, but even in the complete

solution 30 percent of the original dry matter was still to be found in the seed.

Percentage of Dry Matter Lost

The percentage of dry matter lost has been discussed on pages 215–217. The graphs (fig. 34, *A* and *B*) serve to strengthen the conclusion

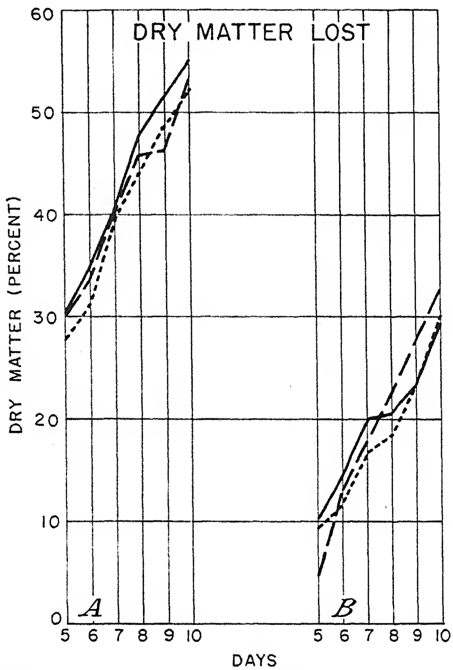

Figure 34.—Percentage of original dry weight of feeds not recovered in seed residue and seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

that this factor was not greatly influenced by the nature of the culture solution and to bring out the fact that the percentage of dry matter lost was very much higher in all solutions at 90° F. than at 69°. There is some evidence that the greatest percentage of dry matter was lost in distilled water at the higher temperature and in the complete solution at the lower temperature.

Germination

Neither the cultures nor the temperatures had any effect on germination percentage, which was 99 for the entire experiment. Although germination was lowest in distilled water and highest in the complete solution, the difference between these two solutions was not significant ($\chi^2=0.04$). The distribution of germinated seeds is given in table 10.

TABLE 10.—*Distribution of germinated seeds in the solutions and temperatures shown*

Temperature (° F.)	Solution			Total
	Distilled water	Eaton's	Calcium	
69	294	299	298	891
90	296	298	295	889
Total	590	597	593	1,780

DISCUSSION

It is evident from these experiments that maize seedlings grown in the dark are benefited by certain nutrient salts, and further that the kind of salts determines the part of the seedling to be benefited. The action of the salts is reflected not only in the total amount of dry matter moved into different parts of the seedling but also in the rate of its movement. Seedlings grown in distilled water, for example, have mesocotyls heavier per unit of length than those grown in the several nutrient solutions, a condition which indicates a retarded movement of dry matter along the axis. In distilled water also the leaves appear later and do not reach, in a given time, the size of those in the salt solutions.

Bonner (*1*) measured the growth, by means of curvature, of excised coleoptiles of *Avena* when immersed in various solutions. He found a more rapid growth in an acid medium (pH 4.1) and reached the following conclusions:

> * * * It would appear then that there are at least two ways connected with growth substance in which the growth rate of an *Avena* coleoptile can be increased. The first is the way in which it is done in the normal plant, by an increase [in] the total amount of growth substance which is present. Only a small portion of this will be in the active non-dissociated form at the pH of the plant, but by increasing the total amount present, the amount present as non-dissociated acid will also be increased. The second way in which the growth rate may be increased is by conversion of some of the inactive growth substance salt already present in the plant into the acid. This is what occurs in the "acid growth" reaction, when the cells of the plant are actually made more acid, and the ionization of the growth substance reduced.

Thimann and Schneider (*7*), using peeled excised coleoptiles of *Avena* and stems of *Pisum* floating in the solution, demonstrated an increased growth for various concentrations of potassium chloride.

No attempt was made in any of the experiments herein reported to maintain the salt concentrations present at the start. The seedlings were enclosed in tin cylinders, which were unopened from the time of planting until the plants were measured. In consequence, growth in the salt solutions may have been checked either by the exhaustion of the required salts or by an excessive concentration through water loss. Although the tin cylinders retarded the loss of water from the system, the actual quantity of condensed water on the inside of the containers and on the plants themselves constituted a loss sufficient to bring about a real increase in the concentration of the culture solutions.

Steward and Preston (*6*) studied the effect of salt concentration on the metabolism of potato disks and reached the following conclusions:

> 2. The effects of salt concentration on starch hydrolysis are slight. The tendency is for increased concentration of potassium salts to decrease and calcium salts to increase starch hydrolysis. This is the converse of the more important effect of salts on all the processes which involve oxidation.

3. Increased external concentration of potassium salts increases respiration and all other reactions which are favored by oxygen, whereas corresponding concentrations of the calcium salts with a common anion depress these processes.

4. The salt concentrations which induce high respiration do not produce high sugar content. High rates of respiration and lower sugar content obtain in the tissue exposed to strong potassium salts and the converse is true of the disks treated with calcium salts. Sugar concentration does not determine respiration.

5. The effective ions of the salts are the cations. The specific effects of the cations are accentuated by the anions and these, like the contrast between the effect of potassium and calcium salts at the same equivalent concentration, are influenced by the anions in the order $NO_3 > Cl > Br > SO_4$ which is also the order in which they influence absorption of a common cation.

6. The effects of salts on respiration are closely connected with their effect on protein synthesis from stored amino acids. Potassium salts stimulate, calcium salts depress both processes.

The behavior of these potato disks may be closely analogous to the processes that take place in maize seedlings growing in the dark. Chemical analyses were not made of the seedling parts, but Frey-Wyssling and Blank (*4*) have shown, with the coleoptile of maize, that water-soluble and protein nitrogen migrate from the seed into the coleoptile as long as it is growing.

In potato disks protein synthesis is depressed in calcium solution relative to potassium salts, and if this process holds true for maize it might explain the retarded growth of coleoptiles and leaves in the calcium solutions tested. However, it should be noted that there is an interaction of solution with temperature of such a nature that the calcium salts at 69° F. have relatively less effect on leaf size than at 90°.

The development of roots, as measured by their dry weight, is unaffected by Eaton's solution (tables 6–8 and figs. 13 and 22). However, the solution of calcium salts is obviously detrimental. White (*8*) showed, with excised root-tip cuttings of wheat growing in Upsenski solution, that growth could be improved by lowering the calcium by as much as 50 percent.

Naylor (*5*) studied the effects of temperature, calcium, and arsenous acid on seedlings of *Poa pratensis* growing in the light and reached the general conclusion that calcium in large quantities is detrimental to young seedlings. Once the seedlings are established, however, calcium stimulates rapid growth. The detrimental effect, at least in the early seedling stages, may be the result of root inhibition. Certainly, with maize, in those cases where seedling survival depends upon a maximum extension of the mesocotyl, calcium is beneficial.

Day (*2*) showed, with *Pisum sativum*, that plants starved for calcium are dwarfed, and apparently this element plays an important role in the stem elongation.

The morphology of the maize seedling is such that the mesocotyl has an upper limit of length, as does also the coleoptile. Under ordinary conditions, neither of these organs reaches the maximum length. To a certain degree they elongate together, though in the early stages the rate of elongation is more rapid in the mesocotyl. The elongation of the coleoptile continues after that of the mesocotyl stops. By the time the first leaf is exserted from the coleoptile, extension of the mesocotyl is complete.

Under all except optimum conditions, the mesocotyl does not reach its full possible length and the leaves begin growth. As the leaves are not exserted from the coleoptile until the mesocotyl has stopped elongating, conditions that speed the growth of this organ speed the appearance of the leaves. Once the leaves have begun to increase in

size rapidly, the mesocotyl shrinks somewhat in length and noticeably in diameter. This shrinkage in size is accompanied by a loss of dry matter.

Where two solutions are used, one of which stimulates the development of the mesocotyl and the other the development of the leaves, it is evident that in the one case leaf growth will be delayed by the developing mesocotyl and in the other case the full length of the mesocotyl will not be reached because of the nutrition requirements of the rapidly expanding leaves.

Although these experiments were terminated before the translocation of dry matter was complete, there is little reason to believe that the leaves in the calcium solution would ever have reached the dry weight of those in the complete solutions. The inhibitory effect of calcium on leaf development, as compared with the more complete solutions, cannot be attributed entirely to the delay in leaf growth brought about through the stimulation of the mesocotyl by this element.

SUMMARY AND CONCLUSIONS

Maize seedlings grown in the dark were benefited by certain salts in the culture medium.

These salts increased the rate of transfer, and the total quantity translocated, of the dry matter stored in the seed.

Calcium salts stimulated the elongation of the mesocotyl but did not produce the longest or heaviest leaves.

Leaf growth was stimulated at the expense of the mesocotyl by all except calcium salts.

Coleoptiles and leaves apparently reacted to the same solutions.

Dry weight of roots was not increased by the salts used and was depressed by calcium salts.

The effects produced by the salts used were dependent on temperature in the range 69° to 90° F. In certain respects calcium salts are relatively less effective in stimulating growth at the lower temperature.

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AN APPARATUS FOR STUDYING RESPIRATION OF AZOTOBACTER IN RELATION TO THE ENERGY INVOLVED IN NITROGEN FIXATION AND ASSIMILATION [1]

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INTRODUCTION

The discovery of *Azotobacter* by Beijerinck (*1*) [3] in 1901, encouraged a vast amount of work on the physiology on this group of nitrogen-fixing bacteria. Since the free-energy data published by Lewis and Randall (*10*) appeared, the interest of other investigators (*5, 6, 7, 12, 13*) in these organisms has centered on the energetics and efficiency of the nitrogen-fixation process, the efficiency of growth, and also on the efficiency of oxygen utilization.

Studies on the rate of respiration of *Azotobacter*, while being subjected to a wide range of carefully controlled environmental conditions, were started in August 1929, at Berkeley, Calif., and continued during the following 2 years. In this paper the apparatus and methods used are described and certain of the results of the investigations are presented.

The chemical reactions catalyzed by *Azotobacter* to obtain the necessary energy for its metabolic processes vary greatly. Under aerobic conditions the oxidation of glucose to carbon dioxide and water is a typical reaction which is catalyzed by these organisms. It may be written, using the terminology of Lewis and Randall (*10*),

$$C_6H_{12}O_6+6O_2=6CO_2+6H_2O;\ \Delta H=-674{,}000 \text{ cal.}$$

The rate at which this reaction is carried out may be considered a direct measure of the metabolic activity of the culture. Consequently the rate of respiration of a culture of *Azotobacter* under different environmental conditions can be determined by measuring the rate at which this reaction proceeds. This rate may be measured in either of two ways: (1) by measuring the rate of disappearance of the reacting substances, such as the rate of absorption of oxygen; (2) by measuring the rate of formation of the end product, carbon dioxide, or the heat of the reaction.

As long as the organisms are utilizing free oxygen, as opposed to combined oxygen, in the reactions catalyzed for a source of energy, a measure of either the rate of oxygen consumption or the rate of carbon dioxide production or the rate at which heat is evolved will give an accurate index to its metabolic activity.

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[2] The author is indebted to G. N. Lewis, Merle Randall, D. R. Hoagland, A. R. Davis, and C. B. Lipman, of the university staff, and to F. C. Steward, of the Department of Botany, Birkbeck College, University of London, for interest shown in the work and for valuable suggestions and criticisms.

[3] Italic numbers in parentheses refer to Literature Cited, p. 248.

However, there is evidence (*2*, *9*) that *Azotobacter* catalyzes energy-liberating reactions not involving free oxygen under anaerobic or partly anaerobic conditions. This has been confirmed by the author. Under such conditions, the rate at which the reaction proceeds does not give a complete measure of the metabolic activity of the culture or an indication of the total energy liberated. Consequently, experiments at low oxygen tensions in which only the rate of free oxygen utilization or the rate of carbon dioxide production is measured would be of little value in determining the efficiency of growth or of oxygen utilization per cell or the efficiency of the nitrogen-fixation process.

CRITIQUE OF METHODS

Most of the earlier work on the physiology of *Azotobacter* with respect to oxygen utilization and carbon dioxide production was done with a qualitative outlook and was carried out with few if any precautions taken to keep the partial pressure of oxygen and carbon dioxide in the culture constant. Nor was this work done in such a way that the reactions catalyzed by the organisms would proceed at a maximum rate under the conditions imposed. The methods in these respiration studies were in general inadequate to give an accurate picture of the metabolic activity of *Azotobacter* over relatively short periods under carefully controlled conditions. This critique of the methods may be said to be especially valid where respiration studies were conducted with partial pressures of oxygen which deviated considerably from that in air.

It will be desirable at this point to discuss certain methods used by later investigators. A consideration of their results will be postponed until after the discussion of the experimental results presented in this paper.

Bonazzi (*2*) made a study of the oxygen consumption of *Azotobacter*. In one experiment 2,566 cc. of air was confined over 100 cc. of an inoculated culture medium and incubated for 52 days. From table 4, page 339, of Bonazzi's paper, it is evident that the partial pressure of the gases in the system varied greatly. In this experiment, the concentration of CO_2 in the air above the culture increased 160-fold during the experiment. At the same time the partial pressure of oxygen decreased to one-twentieth. The composition of the medium with respect to certain ions was similarly changed. There were 44.36 mg. of CO_2 dissolved in the 100 cc. of medium at the end of the experiment, making the medium 0.01 molal with respect to carbonic acid. As there were only 0.02 gm. of K_2HPO_4 (0.001 molal) to serve as a buffer, this concentration of carbonic acid, which would not be affected much by the other salts present, would bring the pH of the medium to about 5.5. There would result approximately a 300-fold change in the hydrogen-ion concentration.

It is evident that the question of equilibrium was not taken into consideration in these experiments. During the first few hours in such experiments the available oxygen and carbon dioxide concentration might have permitted a rapid aerobic respiration, but the conditions would soon have become more favorable for anaerobic respiration than for aerobic respiration.

Meyerhof and Burk (*13*) and Lineweaver, Burk, and Horner (*11*) have made an extensive study of the physiology of *Azotobacter* by the use of the Warburg technique (*15*).

The total volume of the apparatus used (*4, 6*) was about 12 to 16 cc. For the removal of carbon dioxide, these investigators relied entirely upon the normal rate of diffusion of the gas from an alkaline medium (buffered usually at pH 7.3) into the confined atmosphere above, aided only by a gentle rocking of the entire apparatus.

The total gas-liquid interface from which the gas exchange must take place was approximately 6 $cm.^2$, or 3 $cm.^2$ of gas-liquid interface per cubic centimeter of medium (*4*). It was then necessary for the carbon dioxide that did reach the gas phase to diffuse down into a small chamber (approximately 0.8 cm. in diameter) containing 0.2 cc. of potassium hydroxide. The area of the interface between the air and potassium hydroxide was extremely small, being approximately 0.5 $cm.^2$ The gas-liquid interface was increased, however, by shaking the manometers at the rate of 120 cycles per minute with an amplitude of 3 cm.

The environment to which Meyerhof and Burk (*13*) and Lineweaver and associates (*11*) subjected the cultures of *Azotobacter* in the Warburg apparatus was carefully controlled and reproducible. It does not follow, however, that the environment with respect to gaseous exchange was such as to allow respiration to proceed at a maximum rate, especially at the higher oxygen pressures.

EXPERIMENTAL METHODS

THE CALORIMETER

The apparatus designed and built for these studies was such that the metabolic activity of a culture could be determined accurately by measuring simultaneously the rate of heat and carbon dioxide production of *Azotobacter* over the entire range of oxygen pressures during nitrogen fixation and nitrogen assimilation.

The differential calorimeter built by Randall and Rossini (*14*) formed the nucleus of the apparatus used in these studies.

The calorimeter consisted of three identical units, as shown in figure 1. Each unit was mounted separately on the under side of a steel plate (*A*). A unit consisted of an electric heating coil (*B*), a cooling coil (*C*), a stirrer (*D*), and one leg of a thermal (*F*), all of which fitted down into a 1,500-cc. vacuum flask (*E*). The vacuum flasks for the three units were held in position by placing them in separate heavy copper cans (*G*), which were then bolted to the steel plate (*A*).

Balsa wood blocks (*H*), and cork supports (*I*) were used to insulate the vacuum flasks from the copper cans and the steel plate.

The entire calorimeter was submerged in an oil bath.

In order for *Azotobacter* to grow normally in the vacuum flask of unit 2 of the calorimeter, it was necessary to build the heating and cooling coils of glass. For the heating coil, a length of manganin wire having a resistance of 9 ohms was coiled and drawn through a thin-walled pyrex U tube. The tube was then filled with oil and sealed, care being taken to leave a small air space to allow for expansion. The cooling coil consisted of several loops of thin-walled pyrex tubing.

A metal bearing was designed for the stirrer so that it would turn freely and yet not leak under pressure. Although the bearing was built to withstand a pressure of 30 mm. of mercury without leaking,

the pressure in the calorimeter never varied more than 4 mm. from atmospheric pressure during an experiment. The mouth of the vacuum flask of unit 2 was sealed with pure gum rubber (*L*) to prevent air from passing from the vacuum flask to the copper can. The three-vaned stirrer (*D*), made of pyrex, was electrically driven at a constant rate of 130 r.p.m.

In order to obtain a direct measure of the rise in temperature produced by the bacteria, it was necessary to hold unit 1, which contained one leg of a multiple-junction thermal (F_{50}–F_{20})[4], at a constant temperature. A search of the literature was made to find some salt with a transition temperature near 25° C. which would serve to hold unit 1 at a constant temperature. It was found that the transition temperature of $Cu(NO_3)_2 \cdot 6H_2O$ to $Cu(NO_3)_2 \cdot 3H_2O$ is 25.28° C.

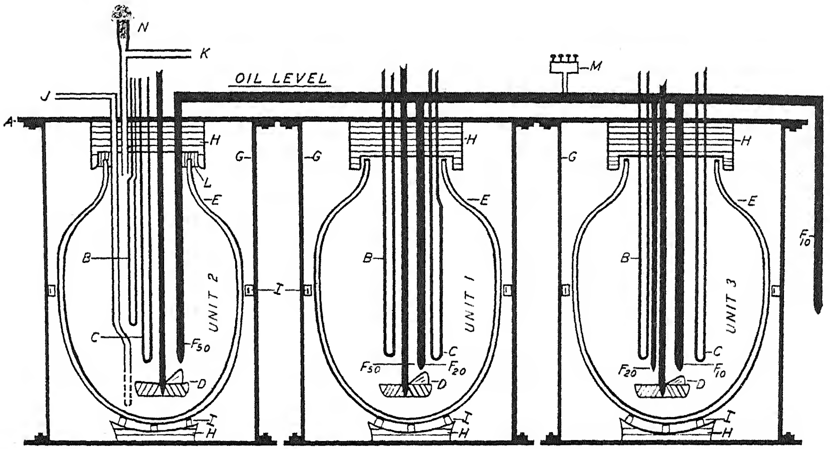

FIGURE 1.—Cross section of the reconstructed calorimeter: *A*, Steel plate; *B*, electric heating coil; *C*, cooling coil; *D*, stirrer; *E*, vacuum flask; *F*, thermals; *G*, heavy copper cans; *H*, balsa wood blocks; *I*, cork supports; *J*, pyrex glass air inlet tube; *K*, exit tube; *L*, gum rubber seal; *M*, terminals; *N*, culture medium inlet.

Other properties of the salt made it ideal for this purpose. Consequently, to the vacuum flask of unit 1, containing 1,000 cc. of a saturated solution of copper nitrate at 25.28° C., was added 800 gm. of an equal mixture of $Cu(NO_3)_2 \cdot 6H_2O$ and $Cu(NO_3)_2 \cdot 3H_2O$. The stirrer of unit 1 was permanently disconnected to prevent the addition of heat to the unit by this means. The oil bath in which the whole assembly was submerged was maintained at 25.28° C. Consequently, no temperature gradient existed between unit 1 and the bath.

Unit 3 of the calorimeter served indirectly to determine the temperature difference between unit 1 and the bath. Consequently, a slight drift in temperature of this unit was not objectionable. Unit 3 was filled with 1,200 cc. of water and at the beginning of each experiment was brought to the temperature of the bath and the stirrer was disconnected. Observations revealed that unit 3 would not vary as much as 0.005° C. for a period of a week. The slight temperature

[4] When the potential difference between units 1 and 2 was measured, the thermal *F* in unit 1 served as one leg of a 50-junction thermal. However, when the potential difference between units 1 and 3 was measured, it served as one leg of a 20-junction thermal.

variations of the oil bath were practically eliminated before they could affect the temperature of the contents of the vacuum flasks of the three units. Unit 3 and the oil bath each contained one leg of a 10-junction thermal (F_{10}). By connecting the potentiometer with the proper terminals (*M*), the temperature difference between units 1 and 2, 1 and 3, or 3 and the oil bath, could be accurately determined at any time. Frequent readings of the 20- and 10-junction thermals showed that the temperature drift of units 1 and 3 was insignificant. The drift in temperature of unit 1 was also determined with the aid of a platinum resistance thermometer which was inserted directly into the unit. The maximum drift, as shown by these two separate methods, was never as much as 0.1 percent of the change produced by the culture over a 5-hour period.

AERATION (GAS-LIQUID INTERFACE)

For *Azotobacter* to carry on its metabolic activities at the maximum rate under the conditions imposed upon it, special precautions were

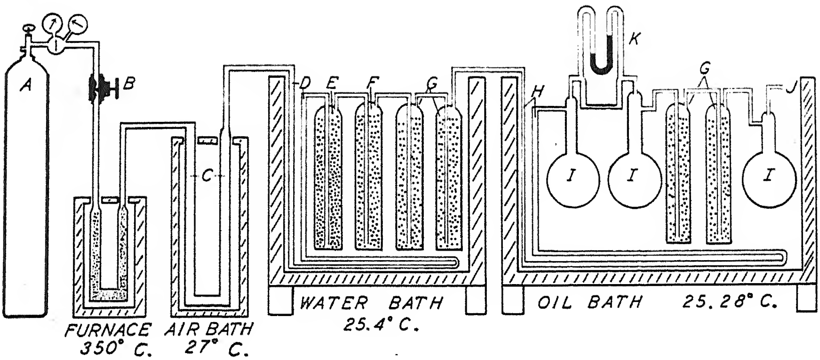

FIGURE 2.—The apparatus used to condition the gas before it entered the calorimeter: *A*, Compressed air tank; *B*, needle valve; *C*, flattened copper pipe; *D*, 15-foot copper tube; *E*, acid tower; *F*, alkali tower; *G*, towers containing distilled water; *H*, copper tube in oil bath; *I*, flasks serving as air cushions; *J*, pyrex tube leading to air inlet *J* of figure 1; *K*, calibrated flow meter.

necessary in order to be certain that the system was in equilibrium with respect to oxygen and that the CO_2 was removed as fast as it was formed. Vigorous aeration of the mechanically stirred culture with small bubbles of the gas is not only effective in sweeping out CO_2 but also furnishes an extremely large gas-liquid interface from which the gas exchange may take place.

Since heat measurements were made on the culture while it was being vigorously aerated, extreme care had to be taken to condition the air or gas properly before it entered the calorimeter in order that the heat change in the calorimeter due to this variable would be small and constant. The apparatus used to properly condition the air is shown in figure 2.

Compressed air tanks (*A*) were pumped to 1,800 pounds pressure with carbon dioxide-free air. The air from these tanks was forced at a constant rate through a 4-foot pyrex tube with the aid of a reducing valve and a specially designed needle valve (*B*). This tube, 45 mm.

in diameter and containing copper oxide, was heated in a furnace to 350° C. to insure the removal of all traces of combustible gases.

The air was further conditioned by passing it in succession through three adjoining constant temperature baths; the first, an air bath, the second a water bath, and finally the oil bath in which the calorimeter (fig. 1) was also submerged. The air, on leaving the furnace, first passed through a flattened copper pipe (fig. 2, *C*), the temperature of which was held at approximately 27° C. by means of the air bath. After this, it passed successively through a 15-foot copper tube (*D*), and acid tower (*E*), and alkali tower (*F*), and two towers (*G*) which contained distilled water. These were all submerged in the water bath at 25.4° C. The air, on leaving the saturating towers in the water bath, was conducted into the oil bath where it took on the final temperature by passing through 31 feet of copper tubing (*H*) immersed in the oil bath. This bath was kept at 25.28° C. and seldom varied 0.01°.

Since the beads in the saturating towers (*G*) tended to cause an irregular flow of air through the flow meter (*K*) [5] and the culture, large flasks (*I*) which served as air cushions were inserted on both sides of the saturating towers (*G*) in the oil bath. The air, on leaving the copper coil (*H*), passed successively into a 4-liter air cushion (*I*), through a calibrated flow meter (*K*), into a 3-liter air cushion (*I*), and finally into the two large bead towers (*G*) which contained distilled water. The air, after having been brought to the proper temperature, took on the last traces of moisture before passing into a 2-liter air cushion (*I*) and then into the vacuum flask (*E* of unit 2, fig. 1) containing the growing culture. The air was conducted to the bottom of the vacuum culture flask by means of the pyrex tube (*J* of unit 2, fig. 1) and forced its way into the medium directly opposite a three-vaned stirrer, through 20 holes in the side of the tube. The holes were spaced evenly over the lower 4 cm. of the tube. The upper holes were approximately 0.4 mm. in diameter, while the center and lower holes were approximately 0.5 mm. and 0.6 mm. in diameter, respectively.

The stirrer was so effective that thermal equilibrium was maintained constantly. The diameter of the circle described by the stirrer was one-third the maximum diameter of the flask. The stirrer moved 8 cc. of liquid per revolution per vane, or approximately 3,000 cc. per minute. The stirrer passed within 3 mm. of the tube delivering the gas. With more than two revolutions per second, six vanes would sweep by the small openings delivering the gas each second. This was very effective not only in breaking up the bubbles but also in distributing them uniformly throughout the medium. It was estimated from observation that between 50 and 75 percent of the bubbles were broken by the cutting action of the stirrer and the vigorous agitation of the medium.

The surface area of the gas supplied by the different rates of aeration was determined by experiment. Air was forced at a constant rate through a 0.5-mm. opening in the side of a glass tube into a vessel containing 1,200 cc. of water. The number of bubbles was counted for a

[5] The flow meter was calibrated by connecting a gas meter to the end of the assembled line. Five determinations of 1 hour each were made covering the range from 9 to 24 liters per hour. These values were plotted and a straight line obtained.

definite period and the total volume noted. The average volume of the bubbles was determined for eight different rates of aeration between 10 and 24 liters per hour. The volume of the bubbles for the different rates of aeration varied from 0.108 cc. per bubble for a rate of 10.8 liters per hour to 0.095 cc. per bubble for 24 litters per hour, with an average of 0.1019 cc. per bubble. The total surface area of gas available to the liquid for gaseous exchange was calculated for the different rates of aeration, using 0.1019 cc. for the average volume of the bubble and the conservative estimate that 50 percent of the bubbles were broken by the cutting action of the stirrer and the vigorous agitation of the liquid. The gas surfaces are not only extremely large but also present a very efficient surface for gas exchange to take place.

The surface area of liquid coming in direct contact with the gas is still of a higher order of magnitude because of the fact that each bubble of gas having an average surface area of 1.055 cm.^{2} rising a distance of 15 cm. (25 diameters) through the liquid would come into

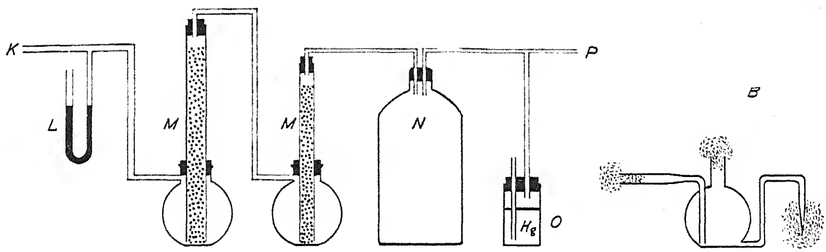

FIGURE 3.—At left, the CO_2 absorbing towers and automatic pressure-regulating apparatus: *K*, inlet tube for air from the growing culture in the calorimeter; *L*, water manometer; *M*, CO_2 absorbing towers; *N*, carboy serving as air cushion; *O*, mercury pressure-regulating device; *P*, end of air line. At right (*B*), flask used to aerate culture with a side arm for aseptic transfer of the culture to the calorimeter.

direct contact with approximately 25 cm.^{2} of liquid surface. Consequently, the surface area of liquid exposed to the gas surface varied from 29×10^5 cm.^{2} for an aeration rate of 10 liters per hour to 48×10^5 cm.^{2} of liquid surface for 24 liters per hour.

The surface of the liquid, being changed continuously by the rapid stirring and vigorous aeration, formed an additional effective gas-liquid interface. Calculations show that the agitation of the liquid by the stirrer alone would move the liquid at a rate sufficient to change the entire surface of the liquid to a depth of 5 mm. 75 times per minute. The air above the culture was completely replaced once every minute with an aeration rate of 18 liters per hour.

CARBON DIOXIDE ANALYSIS

The air on leaving the growing culture in the calorimeter was drawn by suction through two CO_2 absorbing towers which are shown in figure 3, *M*.

Atmospheric pressure, as measured by a water manometer (*L*), was maintained inside the vacuum culture flask despite the number of

towers and flasks in the system. This was accomplished by connecting the end of the air line (*P*) to a constant source of vacuum in series with a 40-liter carboy (*N*) which served as an air cushion. The mercury pressure-regulating device (*O*) automatically maintained the proper negative pressure at all times. The water manometer (*L*) was inserted in the line (fig. 1, *K*) on leaving the culture flask. With a constant pressure from the compressed air tanks forcing the air to the culture flask, and with a constant negative pressure drawing the air from it through the CO_2 towers, it was an easy matter to maintain atmospheric pressure in the culture flask well within 4 mm. of mercury at all times. However, when heat measurements were made, even greater care was taken and the pressure was controlled to within 1mm. of pressure. This was found to be necessary inasmuch as the heat correction for the air varied slightly with the pressure.

The CO_2 produced by the culture was absorbed in the first tower. To make certain that this absorption tower completely removed all CO_2, the air was drawn through a second smaller glass bead tower. The first tower was found to be very efficient in removing all traces of CO_2 from the air since the second tower was used continuously for 200 hours without becoming cloudy.

A weighed amount of barium hydroxide solution was added to the assembled CO_2-free tower and enough CO_2-free water was added so that absorption took place the entire length of the tower. In analyzing for the CO_2 present, the tower was disconnected from the line, the beads and alkali in the tower were washed down into the extraction flask, and the tower was thoroughly rinsed with CO_2-free water. The excess barium hydroxide was then titrated against 0.08 normal hydrochloric acid, with phenolphthalein as an indicator.

To prepare the tower for absorption, the beads were transferred to a porcelain sieve. The beads, tower, and flask were washed with hydrochloric acid, thoroughly rinsed with tap water, with distilled water, and finally with CO_2-free water. The tower was then assembled, the beads added, and immediately sealed. When the barium hydroxide was added, it remained clear, showing that the tower was free from CO_2.

PREPARATION OF CULTURES

Pure cultures of *Azotobacter chroococcum* and *A. vinelandii* were obtained from the American Type Culture Collection.[6] Although the latter was used in most of the work, experiments showed that both gave the same results. In order to be certain that the culture remained pure and did not acquire any unusual characteristics, a second culture of *A. vinelandii* was obtained from the same source 1 year later and carried through the same experiments.

The stock cultures were preserved on dextrose agar slants kept in the ice box. One hundred cubic centimeters of liquid medium in 250-cc. Erlenmeyer flasks was inoculated from the slants. These liquid cultures, incubated at 28° C. and transferred every 2 weeks, were used to inoculate the medium used in the experiments. Every 3 months new liquid cultures were started from the stock cultures kept on ice.

[6] American Type Culture Collection, Chicago, Ill.

The medium used had the following basic composition:

K_2HPO_4	1.0 gm.
$MgSO_4.7H_2O$	0.2 gm.
NaCl	0.2 gm.
$CaCO_3$	0.005 gm.
$FeCl_3$	Trace
$MnCl_2$	Trace
Glucose	10 gm.
Distilled water	1,200 cc.

(The pH was adjusted to 7.1 to 7.2)

The inorganic constituents were added to the distilled water and sterilized in the autoclave at 20 pounds pressure for 30 minutes. Ten grams of glucose were then added and the total weight of medium was made up to 1,195 gm. The medium was then transferred to a sterile flask (fig. 3, *B*) in which the cultures could be aerated with sterile air. This sterile flask, after receiving the adjusted and partly sterilized medium, was autoclaved for 1 hour at 5 pounds pressure.

After the last sterilization, the medium was inoculated with 5 cc. from the liquid cultures, making a total of 1,200 gm. of culture medium. Depending upon the experiment, the content of the flask was either transferred to the sterile vacuum flask (fig. 1, unit 2) immediately, or else inoculated and aerated with st .ile air for 12 to 24 hours at the rate of 20 liters per hour before being transferred to the vacuum flask of the calorimeter.

The aseptic transfer of the medium was accomplished with the aid of the side arm (fig. 3, *B*). The tip of the side arm was placed directly over the entrance tube (fig. 1, *N*). The cotton plug and the cotton enveloping of the side arm were flamed and the flask was lowered until the plug and covering of the side arm came in contact before the flame was extinguished. The flask was then lowered still further, allowing the sharp tip to pierce the cotton covering and also the plug and enter the tube (fig. 1, *N*) of the calorimeter. Sterile air forced the medium into the side arm, through the opening in the side of the tube, figure 3, *B*, and into the sterile calorimeter.

The sterilization of unit 2 (fig. 1) of the assembled calorimeter was effectively accomplished by completely filling it and the entrance and exit tubes with a suspension of calcium hypochlorite. This was stirred from 12 to 24 hours and then siphoned out. The remaining hypochlorite was effectively removed by aseptically filling and draining the calorimeter, together with the entrance and exit tubes, four successive times with sterile distilled water, flasks like that in figure 3, *B*, being used.

After this method of sterilizing unit 2 of the calorimeter was adopted, no contamination was found. All the experiments reported were carried to completion with no evidence of contamination.

To be certain that no contaminating organism was present, cultures were taken from the vacuum culture flask at the end of each experiment, transferred to a sterile medium, and also examined microscopically. Subcultures were also made and examined.

CALIBRATION RESULTS

HEAT CAPACITY

After the entire apparatus was reconstructed and assembled, calibration runs were made to determine the heat capacity of unit 2 which was to contain the growing culture.

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It was found that with the changes in unit 2 (fig. 1) its sensitivity was not affected. Duplicate determinations showed a heat capacity for unit 2 of 64.8 and 64.9 calories per degree. The heat capacity of unit 2 includes that of the vacuum flask, the stirrer, the thermal (*F*), entrance and exit tubes (*J* and *K*), and the cooling and heating units within the flask.

Calibration runs were made on the sterile medium (1,200 gm.) to determine its heat capacity. Two measurements on the same medium

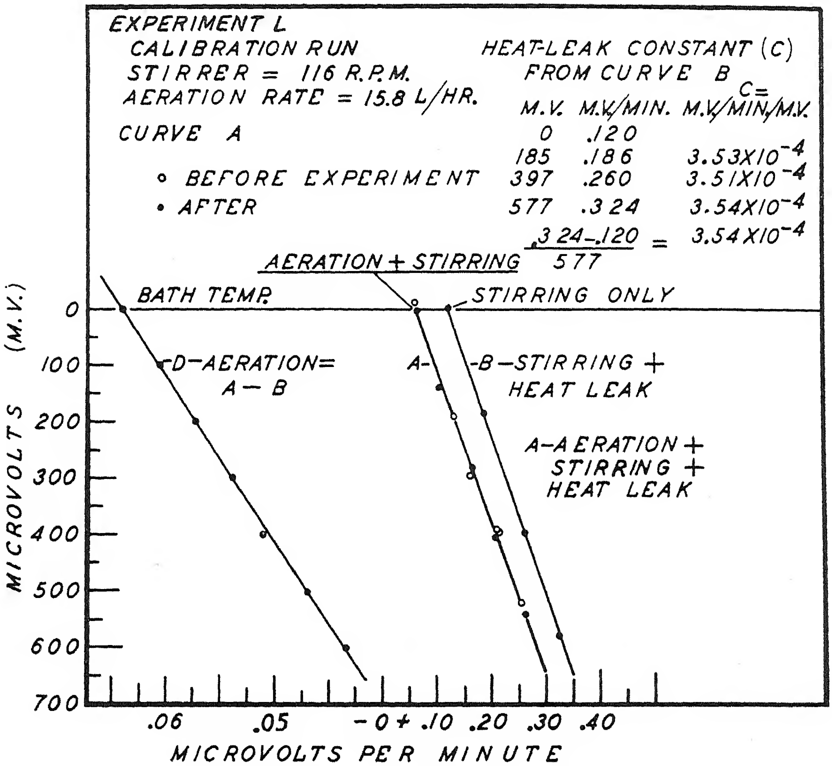

FIGURE 4.—A typical calibration run made to determine the heat-leak constant, stirring correction, and the aeration correction.

gave 1,246.4 and 1,246.5 calories per degree, respectively. The heat capacity of each medium used was determined.

THE HEAT-LEAK CONSTANT

The heat-leak constant *C* equals the calories of heat taken up by the calorimeter from the bath per minute per degree that the calorimeter is below the temperature of the bath.

The heat-leak constant of unit 2 was found to have a slightly lower value than that found by Randall and Rossini (*14*). It was reduced by substituting glass for the copper heating and cooling units, by

eliminating evaporation, and by reducing the heat leak around the stirrer by the installation of the airtight bearing.

The heat-leak constant in all experiments was extremely low, ranging from 2.5×10^{-4} to 4.0×10^{-4} microvolts[7] per minute per microvolt below the bath temperature. When converted, a typical heat-leak constant was 0.0486 calorie per minute per unit of 0.10° C. below the bath temperature. Figure 4 shows a typical calibration run and the calculations.

HEAT CORRECTIONS

The amount of heat added to or removed from the system by the stirring and aeration was determined by special experiments. The constant amount of heat added to the system by stirring was approximately 0.078 calorie per minute for a stirring rate of 130 r. p. m.

The slight cooling caused by the air flow was constant for a given temperature inside the calorimeter and increased very slightly as the temperature of the culture increased. For example, in one experiment the correction of the stirring energy was +2.13 calories, the air correction −2.59 calories, and the heat-leak correction +0.81 calorie. This is a net correction of only +0.35 calorie for a period of one-half hour in a total of 57.50 calories. In this case, the net correction is but 0.61 percent of the total change. However, in some runs where the aeration rate was greater, the net correction was slightly higher. Inasmuch as all heat corrections were determined by special experiments which were accurate to within 10 percent, little error was introduced in the final results.

OPERATION OF CALORIMETER

To measure the heat produced, the culture was cooled from 600 to 900 microvolts (0.4 to 0.6 of a degree) below the bath temperature by circulating cold air through the glass cooling coil (fig. 1, *C*). Liquid air was used to precool the air passing through the cooling coil; consequently the cooling of the culture required not more than 15 minutes. Microvolt readings of the 50-, 20-, and 10-junction thermals were taken every 15 to 30 minutes until the culture reached the temperature of the bath, which required from 2 to 6 hours, depending on the rate of heat production and the degree of cooling. This constituted one run. The microvolt readings were plotted against time. The heat corrections were made and the calories per hour calculated.

CALCULATION OF DATA

Figure 5 shows in detail the data and calculations of one run which is typical of approximately 400 which were made. It represents the measurements made and the procedure followed to obtain each point (calories per hour) on the curves of the experiments which will be presented. The heat measurements were made continuously during each experiment except for the short period of 15 minutes between each run. The length of runs varied generally from 2 to 6 hours, depending upon the rate of respiration and the degree to which the culture was cooled.

[7] One microvolt is equal to 46.185×10^{-5} degrees difference in temperature.

SENSITIVITY OF APPARATUS

Measurements presented in figure 6 show that the apparatus used was so flexible and sensitive that any changes in the rate of respiration of the culture could be detected immediately and measured accurately. The speed with which measurable differences in heat production could be detected when moderate changes in the oxygen tension were made is clearly evident from the figure. Within 5 minutes after the con-

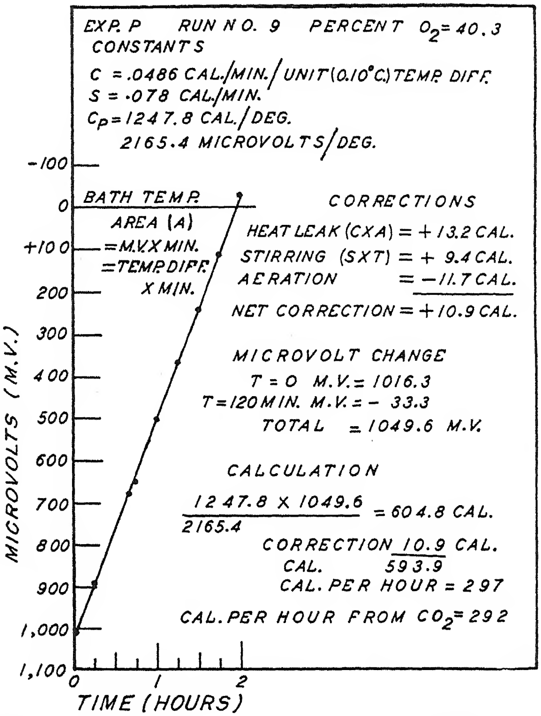

FIGURE 5.—Measurements made and procedure followed in calculating the rate of heat production.

centration of oxygen was changed the temperature response was apparent from the microvolt readings.

In every case where the change made in the partial pressure of oxygen was not more than 100 percent, less than 15 minutes was required for the establishment of a steady state at the new oxygen pressure. This is shown by the fact that the culture would make its total change in the rate of respiration in this short period and then proceed at a constant rate. It is evident from the straight lines obtained over such long periods that conditions were carefully controlled.

Where the partial pressure of oxygen is suddenly reduced to a small fraction of the original pressure, considerable time is required for the culture to reach a steady state at the lower value. In certain experiments the partial pressure of oxygen was suddenly decreased to one-twentieth and in certain experiments to as low as one two-hundredths

of the initial value. It is obvious that considerable time would be necessary even with vigorous aeration for the culture to reach a steady state of respiration. The author determined the minimum time required to reduce the concentration of oxygen from 18 to 0.1

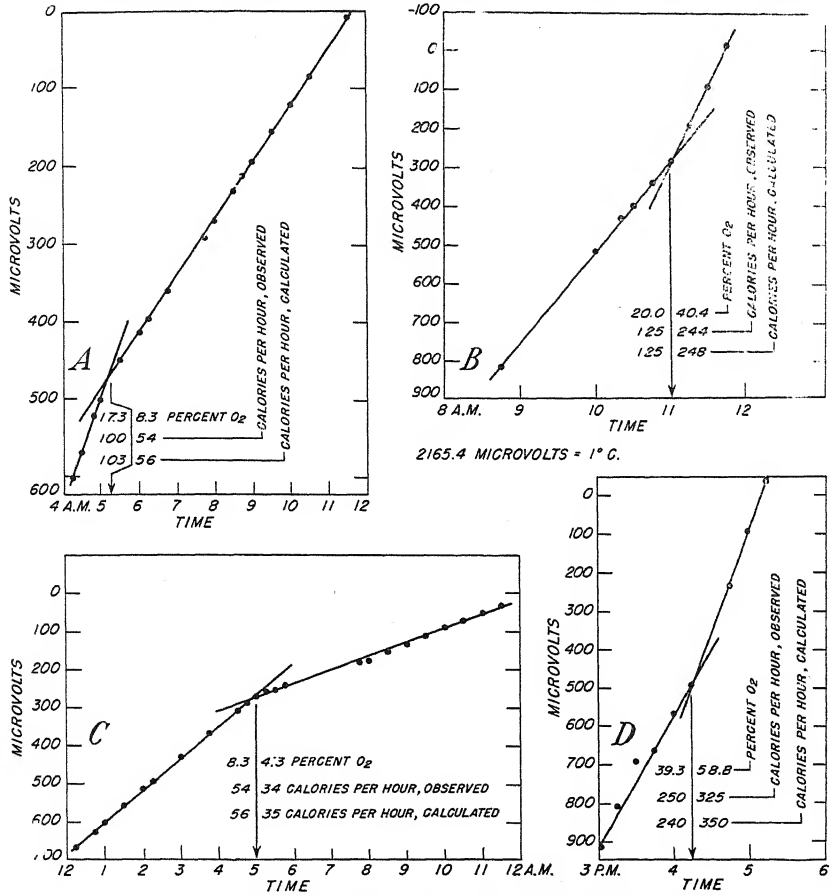

FIGURE 6.—Graphs showing the speed with which changes in the rate of respiration of the culture (caused by moderate changes in the partial pressure of oxygen) could be detected with the calorimeter used. The arrow indicates the time the new gas mixture reached the culture. The figures separated by the arrow show the percent of oxygen used and the rate of respiration both calculated and observed. *A*, Experiment N, runs 4 and 5; *B*, experiment R, runs 7 and 8; *C*, experiment N, runs 5 and 6; *D*, experiment O, runs 14 and 15.

percent in the culture by aerating it with a gas mixture containing 0.1 percent oxygen until the rate of respiration became constant.

In one experiment a culture that was free from combined nitrogen was inoculated and aerated for 48 hours with air. The culture was then transferred to the sterile calorimeter, as described, and aerated with a gas mixture containing 18 percent oxygen until the rate of respiration, as measured by both the heat and CO_2 evolved, became constant at 105 calories per hour. Nitrogen containing 0.1 percent oxygen was then bubbled through the well-stirred medium at the rate of 14 liters per hour. Heat and CO_2 measurements were made to

determine the time required for the culture to reach a constant rate of respiration with the new gas mixture. The data are shown in figure 7*A*.

The heat measurements show that 15 minutes after aeration was begun with nitrogen containing 0.1 percent oxygen the rate of respira-

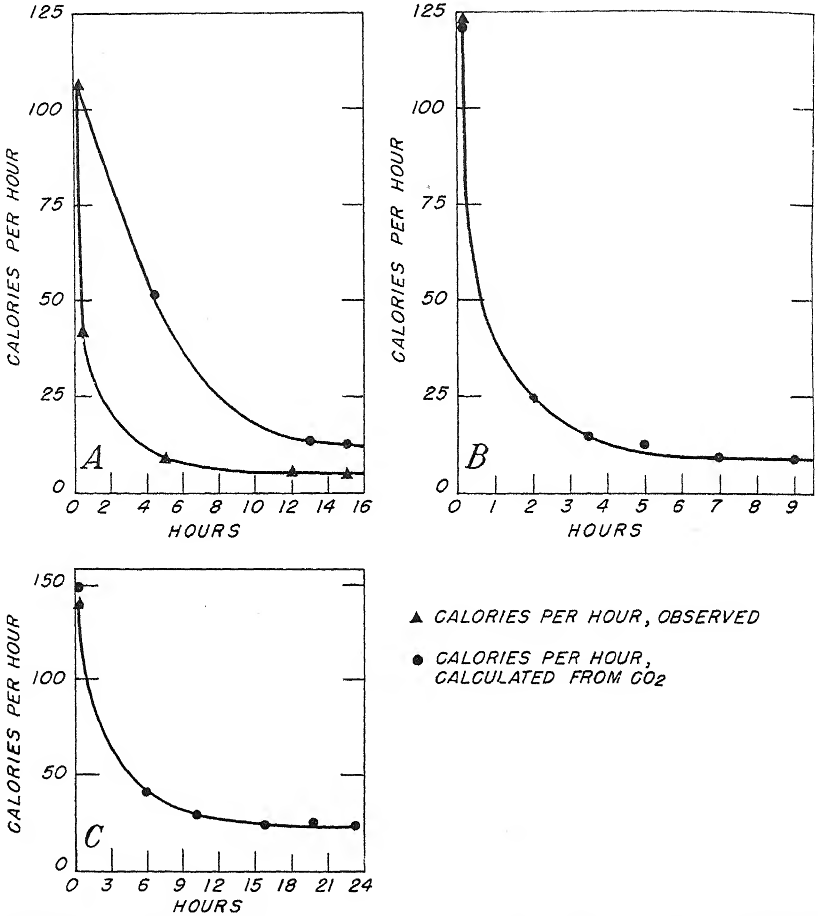

FIGURE 7.—The time required for cultures to reach a steady state with respect to the rate of respiration at the new oxygen pressure when the partial pressure of oxygen is suddenly decreased to a small fraction of the initial pressure: *A*, N_2 fixation; *B*, NH_4 assimilation; *C*, NO_3 assimilation.

tion dropped to only 42 calories per hour, which represents a 60 percent decrease in the first 15 minutes. After 5 hours, the rate of respiration was 4 percent greater than at 15 hours. The rate of respiration did not reach a constant value until at least 10 hours after the 0.1 percent oxygen-gas mixture reached the culture. The final CO_2 measurements at 13 and 15 hours confirm the heat measurements. The

obviously high CO_2 point at 5 hours was caused by a slight mixing of the last portion of CO_2 produced at 18 percent oxygen and that produced at 0.1 percent. The mixing took place in a 20-liter air cushion through which the gas passed after leaving the culture on its way to the CO_2 absorbing towers. This air cushion was removed after this test.

In another experiment, a culture assimilating the ammonium ion was allowed to reach a constant rate of respiration (125 calories per hour) at 20 percent oxygen. The culture was then suddenly subjected to nitrogen gas containing 0.1 percent oxygen and the rate of respiration was determined continuously until it reached a constant value. The data obtained are shown in figure 7, *B*. Here, again, the rate of respiration dropped very rapidly during the first 2 hours. It required 7 hours, however, for the culture to reach a constant rate of respiration.

A similar experiment was carried out in which a culture was assimi-

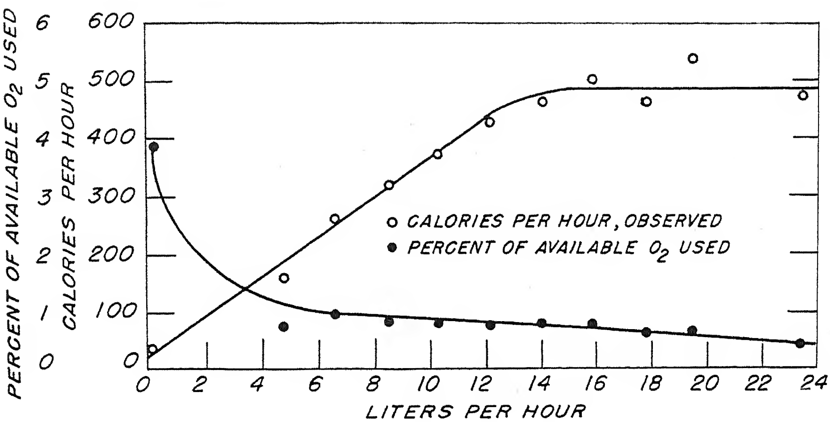

FIGURE 8.—The rate of respiration of *Azotobacter* (in a nitrogen-free medium) with 90 percent oxygen as influenced by the rate of aeration.

lating the nitrate ion. The culture, respiring at a constant rate of 150 calories per hour in 20.4 percent oxygen, was suddenly subjected to nitrogen containing 1 percent oxygen and the rate of respiration was followed continuously until it reached a constant value. The data are shown in figure 7, *C*. Fifteen hours were required for the culture to reach a steady state after the partial pressure of oxygen was reduced to one-twentieth of the initial value.

It is evident that when the partial pressure of oxygen in a culture is suddenly decreased as much as one two-hundredth of the initial pressure considerable time is required for the culture to reach a steady state at the lower oxygen tension even when it is aerated vigorously and stirred mechanically.

RATE OF AERATION

To demonstrate that the gas-liquid interface supplied adequate oxygen at all times and effectively removed the CO_2, the following experiment was carried out: A culture of *Azotobacter*, fixing nitrogen was aerated at different rates varying from 0 to 23.5 liters per hour

with 90 percent oxygen. All the gas passed through the medium which was also mechanically stirred at the normal rate. The rate of heat production was measured for each aeration rate and the oxygen consumed was calculated from the heat produced. The percentage of available oxygen utilized was calculated and is shown by the lower curve of figure 8. The rate of respiration at the different rates of aeration is shown by the upper curve.

The data show clearly that an adequate gas-liquid interface is absolutely necessary if respiration is to proceed at a maximum rate. It is seen that a gas-liquid interface supplied by an aeration rate of 14 liters per hour was required to remove the CO_2 and supply the necessary oxygen for a maximum rate of respiration. From the lower curve it is evident that the culture did not suffer from lack of oxygen when the aeration rate was 6 liters per hour, yet the increased gas-liquid interface resulting from the increased rate of aeration caused an increase in the rate of respiration. However, a 67-percent increase in the rate of aeration above 14 liters per hour with a corresponding increase in the gas-liquid interface did not increase the rate of respiration. This shows definitely that at 14 liters per hour the culture had reached a steady state with respect to the rate of respiration. In other experiments with oxygen concentrations below that of the air, the rate of aeration was in many cases doubled with no increase in the rate of respiration.

RESULTS WITH CALORIMETER

If *Azotobacter* oxidizes glucose to CO_2 and water, without the accumulation of intermediate or partly oxidized compounds, the CO_2 evolved should give a direct measure of the amount of glucose oxidized and the oxygen consumed. One can then calculate how much heat should be liberated by the amount of CO_2 evolved. This amount of energy should be equal to that actually measured in the calorimeter. The amount of energy used for the nitrogen-fixation process is probably insignificant as compared to that liberated as heat.

In order to study the rate of respiration of a culture of *Azotobacter* from the time of inoculation, the calorimeter was assembled and carefully sterilized with a heavy suspension of calcium hypochlorite as described. The sterile medium was inoculated and immediately 1,200 gm. was transferred aseptically to the sterile vacuum flask of unit 2 of the calorimeter. Aeration with 19 percent oxygen was begun at once and heat and CO_2 measurements were made continuously for 96 hours. The results of this experiment are shown in figure 9. The measured heat in calories produced per hour and the amount of heat that should have been produced during the same period as calculated from the CO_2 evolved are plotted against time.

The rate of air flow through the culture in this experiment was 14 liters per hour, which removed the CO_2 as fast as formed. Under these conditions only 0.48 percent of the available oxygen was utilized. As the source of air supply was from compressed air tanks, the partial pressure of oxygen in the system was constant at 0.19 atmosphere throughout the experiment.

The close agreement that exists between the measured and the calculated heat evolved during the entire experiment shows that the glucose was primarily oxidized to carbon dioxide and water (and of

course to the products of cell growth and the other normal products of the reactions of the cells) without a measurable accumulation of intermediate products. It is also evident that under these optimum aerobic conditions, and at this concentration of oxygen, no anaerobic respiration took place, with the result of a respiration quotient of unity.

For the first 8 hours, little or no growth took place. From the eighth to the thirty-sixth hour, the number of living organisms increased rapidly, thus accelerating the rate of heat and CO_2 production. After the thirty-sixth hour, the rate of respiration became constant and remained so to the end of the experiment. Here the number of living organisms was constant, as indicated by the constant rate of heat and CO_2 production. The curve, moreover, shows clearly the growth phases discussed by Buchanan (*3*).

The results reported in figure 9 serve admirably to show what changes may occur in the efficiency with which the cells utilize oxygen,

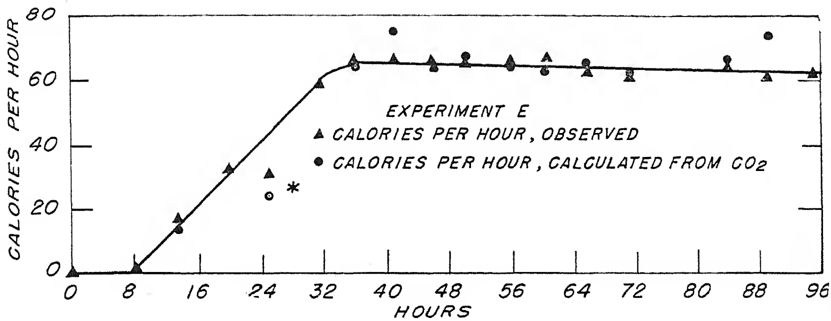

FIGURE 9.—The rate of respiration of *Azotobacter* (while fixing nitrogen) from the time of inoculation, under a partial pressure of 0.19 atmosphere of oxygen. The two points designated by the asterisk were obtained while the mechanical stirrer was being repaired. This decrease in the rate of respiration indicates the absolute necessity of adequate stirring as well as vigorous aeration.

and how the efficiency varies as the age of the culture increases. From inoculation to the thirty-sixth hour, the number of living cells rapidly increased, as shown on the graph by the increasing rate of heat and CO_2 production. During this period, the utilization of oxygen per cell was very high, though not necessarily higher than when the number of living cells became constant. After the thirty-sixth hour, the number of living cells apparently reached a maximum and remained constant throughout the experiment, as determined by CO_2 and heat measurements.

Although the number of living cells remained constant, new cells were rapidly being formed. (Direct evidence supporting this statement is presented in detail in a later paper (*8*).) This increase in the new cells was offset, however, by the old cells dying at the same rate. During this period (thirty-sixth to ninety-sixth hour) when both the number of living cells and the rate of oxygen consumption (measured by both CO_2 and heat production) remained constant, it appears that the efficiency of oxygen consumption per cell is constant and does not decrease with age.

Evidence that only the living cells were responsible for the oxygen consumed and the CO_2 evolved was obtained by measurements before and after the culture was killed with mercuric chloride. In one experiment, the observed heat and the heat calculated from the CO_2 evolved were 47 and 44 calories per hour, respectively. Upon the addition of mercuric chloride to the culture, the observed heat production fell immediately to zero while the rate of CO_2 production was equivalent to only 7 calories per hour. This apparent slow rate of CO_2 production after the addition of mercuric chloride was easily accounted for by the fact that considerable time was required to remove completely the last traces of CO_2 even with vigorous aeration and stirring. At the end of 3 hours of aeration no more carbon dioxide was evolved.

DISCUSSION

For a culture of *Azotobacter* to carry on its normal metabolic processes, it must have at all times an adequate amount of oxygen and at the same time the end products must be removed effectively as fast as formed. In certain experiments reported in the literature where few or no precautions were taken to remove the CO_2 or to supply an adequate amount of free oxygen in the proper manner, it is to be expected that the behavior of *Azotobacter* would differ from that in the present experiments.

In view of the fact that a respiratory quotient [8] of unity was obtained in these studies under controlled conditions, a review of certain of Bonazzi's experiments is desirable.

Bonazzi (*2*), in apparent agreement with other workers, reports $CO_2 : O_2$ ratios from three experiments of 1.08, 1.07, and 1.09, respectively. Bonazzi's experiment No. 44 (*2, p. 338*) reports that the air above the medium contained 309.4 cc. of CO_2 and that 286.2 cc. of oxygen were consumed. The apparent ratio $CO_2 : O_2$ is 1.08 as calculated by Bonazzi. However, all the CO_2 produced by the culture was not considered in the calculation. If the partial pressure of CO_2 above the medium had been kept near that in air, practically all the CO_2 dissolved in the medium would have been found in the gas phase. Titration revealed 50.70 mg. or 25.8 cc. of CO_2 in the medium. This makes a total of 335.2 cc. of CO_2 produced by the organisms while only 286.2 cc. of oxygen was consumed. The true ratio $CO_2 : O_2$ should be 1.17 and very probably would have been greater if the incubation period had been longer or the medium more heavily buffered. When the total CO_2 produced in these experiments is considered, it is clearly evident that intramolecular respiration proceeded under such unfavorable conditions.

Meyerhof and Burk (*13*) present data on the efficiency of oxygen utilization as determined by total respiration and introduce the term "QO_2" to represent the total respiration per milligram of dried cells per hour. It was pointed out that the efficiency with which *Azotobacter* cells can utilize oxygen is very high in young cultures, and decreases very rapidly with increasing age of the culture. In these measurements, the dry weight of the total number of cells without

[8] The oxygen consumed was not measured directly but was calculated from the calories of heat evolved according to the reaction mentioned earlier.

distinction between the living and the dead was used in calculating QO_2.

In measuring the efficiency of oxygen utilization, care must be taken to credit the oxygen consumption only to the living cells and not to the total number of cells in the culture. In an old culture the total number of cells present is by no means an indication of the number of living cells.

From these considerations, it is obvious that measurements of the rate of oxygen consumption per living cell over the entire life of the culture mean nothing if the total number of cells is determined, because only the living cells are utilizing the oxygen. From this, the results of Meyerhof and Burk are what one would expect and, in fact, what one might easily predict. It is also obvious that the amount of oxygen consumed per unit of dry weight of cells per hour must decrease since dead cells increase in number almost from the beginning and continue to do so throughout the life of the culture. On the other hand, the number of living cells, which alone consume the oxygen, very soon reaches a maximum and then, after a period, begins to decline.

SUMMARY

A differential calorimeter was rebuilt so that the heat and carbon dioxide liberated by a culture of bacteria could be measured simultaneously over the entire range of oxygen partial pressures from 0.001 to 1.0 atmosphere.

The apparatus was so designed that the culture could be vigorously aerated with any gas mixture. The vigorous aeration, assisted by the rapid mechanical stirring, presented such an enormous gas-liquid interface to the culture that adequate oxygen at the partial pressure in question was insured and at the same time the carbon dioxide was effectively removed as fast as formed.

The heat and carbon dioxide produced could be measured accurately over long periods of time.

The calorimeter was so sensitive that sudden changes in the rate of respiration caused by changes in the partial pressure of oxygen were quickly revealed by the heat measurements.

The experiments presented demonstrate the need of vigorous aeration and stirring to obtain a maximum rate of respiration.

With a constant partial pressure of oxygen approaching that in the air, the growth phases of a culture of *Azotobacter* were accurately followed.

From the carbon dioxide produced and the oxygen consumed (as calculated from the calories of heat evolved) the respiratory quotient of *Azotobacter* was determined when the partial pressure of oxygen was near that in air.

Azotobacter produced the maximum amount of heat possible from the oxygen utilized. This was found to be true even in old cultures, which indicates that the efficiency of oxygen utilization per living cell remains constant with increasing age of the culture.

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RELATIONSHIP OF CERTAIN CHARACTERISTICS OF SEED COTTONS TO GINNING[1]

By W. Sharrott Smith, formerly *junior cotton technologist*, William J. Martin, *associate cotton technologist, Agricultural Marketing Administration*, and Norma L. Pearson, *associate cotton technologist, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

The removal of the lint fibers from cotton seeds by means of gin saws is not purely mechanical in the sense that all variables which may affect the processes are equal for all lots of seed cotton. Ginning performance and efficiency vary not only with the ginning organization and mechanical perfection of the machinery but also with the seed cotton being processed.[2]

The possibility that varietal characteristics may influence the time and energy required to gin unit weights of seed cotton is one of the many problems arising out of the general investigations being conducted by the United States Department of Agriculture to enhance cotton lint quality through selection and breeding, and through improvement of ginning operations.

Differences in ginning performance of several seed cottons were described as early as 1879 by Watson, who was one of the pioneers in cotton ginning research.[3] Watson measured the time and energy consumed in ginning each of a number of widely different cottons. From these studies he found that Indian cotton, Egyptian cotton, and Indian-grown American cotton differed noticeably in their ginning requirements. He states (Vol. 1, p. 61):

> The difference thus shown to exist between the several varieties of cotton in respect of the power consumed in their cleaning can be accounted for in two ways: differences in the strength of the attachment of the fibre to the seed and differences in the roughness of the seed.[4]

Roughness applies to the degree of fuzziness. Watson's statements imply that large, fuzzy seeds remain in the seed roll longer and retard the action of the gin saws.

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[1] Received for publication June 25, 1942. The cotton varieties studied were grown at Stoneville, Miss., through cooperation with the Delta Branch Experiment Station, and the ginning tests were performed at the U. S. Cotton Ginning Laboratory at Stoneville in cooperation with the Bureau of Agricultural Chemistry and Engineering.

[2] Bennett, C. A., and Gerdes, F. L. effects of gin-saw speed and seed-roll density on quality of cotton lint and operation of gin stands. U. S. Dept. Agr. Tech. Bul. 503, 40 pp., illus. 1936.
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[3] Watson, [J.] F. report on cotton gins, and on the cleaning and quality of indian cotton 2 v. London. 1879.

[4] Watson uses the word "cleaning" in the same sense that the word "ginning" is now used, to describe the process of removing the fibers from the seed.

Federow, [5] a more recent investigator, makes a theoretical analysis of cotton ginning. He considers strength of fiber attachment to the seed coat as one of the principal factors affecting the difference in power required to gin seed cottons. He estimates the strength of fiber attachment to the seed coat to vary from about 2 to 2.7 gm. per single fiber. Federow states (*p. 4*):

> Now, in the last analysis, the power required for ginning depends upon the amount of work each tooth of the gin saw does in pulling the fibers off the seed coat. Each time the tooth enters the roll it will tear, on the average, 100 fibers from the seed. To do this work, 200 to 270 grammes (.444 to .6 lb.) pulling strength must be exerted.

His analysis also contains statements which suggest that the number of fibers per pound of lint, the percentage of lint, and other factors that determine the number of fibers removed from the seed by each saw tooth passing through the roll may affect the time and the energy consumed in ginning.

Although it is a common opinion among cotton ginners that certain lots of cotton may gin more rapidly than others, no definite information is available to show whether there are marked differences in ginning efficiency among American upland cottons. During the years 1936 and 1937, ginning tests were made at the United States Cotton Ginning Laboratory at Stoneville, Miss., to gain information on this point and to ascertain whether certain seed-cotton properties were responsible for such differences as might be found to exist.

MATERIALS AND METHODS

Seed cottons for the ginning tests were obtained from special plantings at the Delta Branch Experiment Station, Stoneville, Miss., in 1936 and 1937. The same 16 varieties were planted both years, but in 1937 another variety, Slick-seeded Acala, [6] was added. In 1936, approximately one-eighth acre of each variety was planted; in 1937 the variety plots were increased to approximately one-fourth acre each. In 1936, the varieties were harvested in 3 pickings each, but the entire yield of each variety was thoroughly mixed before it was divided into triplicate ginning lots. In 1937 each variety was harvested in 2 pickings. The yields from each variety were sufficient for 4 ginning tests for each picking or a total of 8 ginning tests for each variety. In all, 184 lots of seed cotton were tested, 48 (16×3) in 1936 and 136 (17×8) in 1937.

A ginning-test lot consisted of 30 pounds of seed cotton weighed after a period of storage and after all varieties were practically uniform with respect to moisture content. The tests were made on a 10-inch diameter, 20-saw gin with a plain front, operated at a saw speed of 570 r. p. m. A separate electric motor was used to drive the saw mandrel, thus making it possible to measure the number of watt-hours of electrical energy required to drive the saws only during each test. The electrical energy consumed was measured by means of a kilowatt-hour meter of standard type but designed for small readings. It was found that the most satisfactory method of reading the meter was to count the number of revolutions of the aluminum disk mounted on the main shaft of the meter. The disk was marked off into eight

[5] FEDEROW, W. S. COTTON GINNING RESEARCH. Cotton and Cotton Oil News 34 (20): 3-4, 1933.
[6] This cotton is not a commercial variety.

equal divisions and the readings were made to one-eighth of a revolution of the disk. One revolution of the disk was equivalent to 4 watt-hours.

In performing the tests, an effort was made to eliminate or control all variables that might affect the ginning time or the energy consumed except the inherent properties of the seed cottons representing the several varieties. Friction variation was eliminated to some extent by allowing the machinery to run idle each morning before starting the tests until two successive readings of the watt-hour meter indicated that the energy required to overcome the friction of the machinery had reached a practically constant figure. The order of ginning the different varieties in each series was determined by lot.

Before a test was started, 10 pounds of seeds from previously ginned cotton of the variety to be tested were placed in the roll box. A handful from the 30-pound sample of seed cotton was mixed with this seed roll to insure good operation. The breast was then lifted from the saws and the motor was started. When the saws had accelerated to the required speed, and at a signal from the timekeeper, the breast was lowered and ginning commenced. With a stop watch, the timekeeper measured the ginning time which began when the gin breast was lowered to the revolving saws and ended when the seed roll ceased rotating at the end of the test. The timekeeper also counted the number of revolutions of the disk of the watt-hour meter during the same period of time.

The 30-pound seed-cotton sample was fed by hand and at such a rate that the seed roll remained very soft and at approximately a constant density throughout the test. Securing and maintaining a uniform seed roll density for every test was especially important. The personal element entered into this, however, because the density was determined by the gin operator on the basis of observation and "feel." Ginning was continued on each lot of cotton until the seed roll became too soft to rotate. This gave a uniform cleaning of the seeds for different varieties and a definite stopping point for the measurement of the ginning time.

The total watt-hours consumed during each test includes the amount of electrical energy needed to run the machinery alone as well as the amount required to gin the cotton. The net energy that would have been required to turn the unloaded machinery was computed by counting the revolutions of the meter disk while running the machinery idle for 1 minute immediately preceding and for 1 minute immediately following each test, by averaging the two readings, and by multiplying the average by the number of minutes the test consumed. The net energy required to run the machinery subtracted from the total energy gives the net energy consumed in ginning the cotton.

Data obtained from each test were: (1) Time required to gin 30 pounds of seed cotton; (2) watt-hours of electricity consumed in driving the saws during the test, (3) watt-hours of electricity consumed in driving the saws when not ginning for periods of 1 minute before and after each test; and (4) weights of lint, seeds, and mote waste. From these data were obtained: (1) Time required to gin enough seed cotton to produce 10 pounds of lint; (2) net energy in watt-hours consumed in ginning 30 pounds of seed cotton and 10 pound of lint; and (3) lint percentage.

Samples of seed cotton, lint, and seeds were taken for moisture determinations and for laboratory measurements of their respective properties. The lint index, seed index (weight of 100 seeds), and the percentage of fuzz fiber by weight on the seeds were obtained each year for each variety. Measurements of strength of fiber attachment to the seeds were made for each variety of the 1936 crop.

RESULTS AND CONCLUSIONS

The data concerning the time and energy consumed in ginning unit weights of seed cotton were, on the whole, consistent for the 2 crop years. Because of the larger number of replications for the 1937 crop, however, more reliance can be placed upon the data for that year than upon the data for the 1936 crop.

Each year appreciable differences were found between varieties in the average time required to gin them (tables 1, 2, and 3). The varieties tended to behave consistently throughout the series of tests for each year and those varieties that ginned most rapidly in 1936 tended to do likewise in 1937. The average time for 30 pounds of seed cotton from the 16 different varieties ranged from 9.7 to 13.0 minutes in 1936 and from 10.3 to 16.8 minutes in 1937. Slick-seeded Acala, a variety tested only in 1937, required the least time of all, 7.9 minutes.

TABLE 1.—*Mean time required and mean energy consumed in ginning seed cotton and lint from each of 16 varieties grown at Stoneville, Miss., 1936* [1]

Variety	Ginning time required for 30 pounds of seed cotton	Ginning time required for enough cotton to yield 10 pounds of lint	Total energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning enough cotton to yield 10 pounds of lint
	Minutes	*Minutes*	*Watt-hours*	*Watt-hours*	*Watt-hours*
Acala (Rogers)	9.7	9.2	199.6	90.0	84.4
Arkansas No. 17	9.8	10.7	206.0	95.2	103.6
Cleveland (Wannamaker)	11.2	11.0	225.2	95.6	93.6
Cook No. 912	10.6	11.0	211.2	90.4	94.0
Delfos No. 4	12.5	12.9	235.2	95.6	99.6
Deltapine	10.8	9.7	218.0	94.8	85.6
Dixie Triumph No. 759	9.7	10.5	196.0	87.2	94.4
Farm Relief No. 2	13.0	12.7	257.2	110.8	107.6
Half and Half	12.4	10.0	230.8	90.8	114.8
Mexican Big Boll	12.4	12.3	242.8	104.0	103.6
Qualla	11.7	10.0	225.6	100.0	81.2
Rowden No. 2088	12.3	12.5	241.2	109.2	110.8
Startex No. 619	10.7	10.4	211.6	92.4	88.0
Stoneville No. 5	10.9	11.0	218.8	95.6	97.6
Triumph No. 44	10.1	10.8	200.0	89.2	95.6
Wilds No. 5	10.9	12.5	228.8	105.6	120.4
Standard error of difference between means of varieties	.57	.54	8.96	4.67	4.32
Least difference between means of varieties significant at odds of 99 to 1	1.57	1.48	24.64	12.84	11.88

[1] Mean of 3 tests.

TABLE 2.—*Mean time required and mean energy consumed in ginning seed cotton and lint from each of 17 varieties grown at Stoneville, Miss., 1937* [1]

Variety	Ginning time required for 30 pounds of seed cotton	Ginning time required for enough cotton to yield 10 pounds of lint	Total energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning enough cotton to yield 10 pounds of lint
	Minutes	*Minutes*	*Watt-hours*	*Watt-hours*	*Watt-hours*
Acala (Rogers)	11.1	10.2	198.0	64.4	59.6
Acala (Slick-seeded)	7.9	7.6	152.4	56.0	53.6
Arkansas No. 17	12.4	12.4	216.8	69.6	71.2
Cleveland (Wannamaker)	13.6	12.9	233.2	67.6	64.0
Cook No. 912	12.5	12.3	218.0	67.2	67.6
Delfos No. 4	14.0	13.5	242.8	74.0	71.6
Deltapine	13.3	11.3	237.6	72.8	61.6
Dixie Triumph No. 759	11.7	11.5	211.6	67.6	66.4
Farm Relief No. 2	16.8	15.4	297.2	93.6	86.0
Half and Half	13.0	10.3	228.4	72.0	57.2
Mexican Big Boll	13.8	13.1	244.0	76.0	72.4
Qualla	12.9	11.2	224.4	68.8	59.6
Rowden No. 2088	15.7	15.2	272.8	80.8	78.4
Startex No. 619	10.3	9.9	186.0	59.2	56.4
Stoneville No. 5	11.6	10.8	209.2	67.2	62.4
Triumph No. 44	12.3	12.0	220.0	68.8	67.6
Wilds No. 5	13.5	13.9	243.6	76.8	79.2
Standard error of difference between means of varieties	.26	.24	4.53	1.71	1.57
Least difference between means of varieties significant at odds of 99 to 1	.68	.63	11.90	4.49	4.12

[1] Mean of 8 tests.

TABLE 3.—*Results of variance analyses of data concerning the time (minutes) and— energy (watt-hours) required to gin 30 pounds of seed cotton and 10 pounds of lint for 16 varieties grown in 1936, and 17 varieties grown in 1937 at Stoneville, Miss.*

Source of variance	Degrees of freedom	Mean square: Time required to gin 30 pounds of seed cotton	Time required to gin enough cotton to yield 10 pounds of lint	Total energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning 30 pounds of seed cotton	Net energy consumed in ginning enough cotton to yield 10 pounds of lint
1936						
Varieties	15	3.49**	4.05**	912.44**	156.72**	406.96**
Series	2	1.18	.96	547.84*	534.56**	342.96**
Error	30	.49	.44	120.32	32.72	27.92
Total	47	1.48	1.61	391.31	93.65	162.30
1937						
Varieties	16	31.86**	31.68**	8,422.68**	579.00**	634.40**
Harvestings	1	7.06**	43.82**	1,574.88**	64.00*	908.96**
Variety×harvestings	16	.52*	.44*	146.48*	24.00*	21.92**
Series within harvestings	6	1.29**	1.22**	396.04**	101.32**	97.64**
Error	96	.28	.24	82.00	11.68	9.88
Total	135	4.15	4.36	1,103.17	84.74	95.89

*Significant at the 5-percent level; **significant at the 1-percent level.

The ginning time on the basis of 10 pounds of lint gave values practically as widely dispersed and as consistent as the values for 30 pounds of seed cotton (tables 1 and 2). The respective order of varieties in regard to rapidity of ginning, however, was changed considerably because of differences in lint percentages (tables 4 and 5).

Variance analyses of the data show that for each year there are significant differences among the varieties in the time required to gin either 30 pounds of seed cotton or enough seed cotton to produce 10 pounds of lint (tables 1, 2, and 3). In general, a difference of 1.57 minutes in the ginning time for 30 pounds of seed cotton, and 1.48 minutes in the ginning time for 10 pounds of lint, between any two variety means of the 1936 crop, can be considered highly significant (odds 99:1). Similarly, a difference of 0.68 minute in the ginning time for 30 pounds of seed cotton, and 0.63 minute in the ginning time for 10 pounds of lint, between any two variety means of the 1937 crop can be considered highly significant.

TABLE 4.—*Seed-cotton properties of the varieties grown at Stoneville, Miss., in 1936*

Variety	Seed index [1]	Fuzz fibers by weight [2]	Lint [3]	Number of fibers in 30 pounds of seed cotton	Mean strength of fiber attachment to seed [4]	Mean strength of fiber attachment × number of fibers in 30 pounds seed cotton
	Grams	*Percent*	*Percent*	*Hundred thousands*	*Grams*	*Hundred kilograms*
Acala (Rogers)	11.1	9.2	35.3	13,367	1.278	17,083
Arkansas No. 17	10.7	10.1	30.7	9,723	1.397	13,583
Cleveland (Wannamaker)	10.5	14.0	34.3	11,680	1.125	13,140
Cook No. 912	8.8	11.0	32.0	12,715	1.202	15,283
Delfos No. 4	10.2	11.5	32.3	10,780	1.169	12,602
Deltapine	9.4	11.9	37.0	13,040	1.107	14,435
Dixie Triumph No. 759	9.9	10.3	30.7	10,266	1.382	14,188
Farm Relief No. 2	11.8	15.6	34.3	11,260	1.340	15,088
Half and Half	9.1	9.6	41.0	14,785	1.229	18,171
Mexican Big Boll	12.2	12.9	33.7	11,499	1.254	14,420
Qualla	11.3	11.3	39.0	12,790	1.153	14,747
Rowden No. 2088	11.9	13.8	32.7	9,424	1.438	13,552
Startex No. 619	11.0	8.6	34.3	11,213	1.736	19,466
Stoneville No. 5	9.8	11.4	33.0	12,888	1.436	18,507
Triumph No. 44	10.6	11.3	31.0	10,799	1.275	13,769
Wilds No. 5	12.0	10.6	29.3	11,097	1.138	12,628
Average	10.6	11.4	33.8	11,708	1.291	15,041

[1] Average of 10 samples of 100 seeds.
[2] Average of 3 determinations.
[3] Average of 3 ginning tests.
[4] Average of 256 fibers from each of 16 seeds.

Most of the foregoing discussion with regard to time applies equally well to energy. Varieties differed considerably as to the total amount of energy (watt-hours), as well as the net energy required to gin them. The varieties tended to behave consistently throughout the series of tests for each year, and those that required the most energy for ginning either 30 pounds of seed cotton or 10 pounds of lint in 1936 did likewise in 1937 (tables 1 and 2). As in the case of time, the amount of energy required to gin Slick-seeded Acala was least of all.

Variance analyses of the data showed that in both 1936 and 1937 there were statistically significant differences among varieties in both total and net energy consumption (tables 1, 2, and 3). In general, a difference of 12.84 watt-hours in net energy required to gin 30 pounds of seed cotton, or 11.88 watt-hours in net energy required to gin

TABLE 5.—*Seed-cotton properties of the varieties grown at Stoneville, Miss., in 1937*

Variety	Seed index			Fuzz fibers by weight			Lint		
	First picking [1]	Second picking [1]	Average	First picking [2]	Second picking [2]	Average	First picking [3]	Second picking [3]	Average
	Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Acala (Rogers)	13.9	14.0	14.0	8.7	8.8	8.8	37.0	35.3	36.2
Acala (Slick-seeded)	14.4	14.5	14.4	5.2	5.1	5.2	36.0	34.0	35.0
Arkansas No. 17	12.5	12.6	12.6	9.8	9.5	9.6	33.3	31.7	32.5
Cleveland (Wannamaker)	12.5	12.8	12.6	12.3	13.0	12.6	36.7	33.7	35.2
Cook No. 912	10.9	10.7	10.8	10.0	10.3	10.2	35.0	33.0	34.0
Delfos No. 4	11.8	12.2	12.0	11.6	11.4	11.5	35.3	33.7	34.5
Deltapine	10.9	11.0	11.0	10.4	11.3	10.8	40.7	38.3	39.5
Dixie Triumph No. 759	11.4	11.3	11.4	8.5	9.2	8.8	35.0	33.0	34.0
Farm Relief No. 2	14.3	14.7	14.5	14.6	13.7	14.2	37.0	35.3	36.2
Half and Half	11.1	11.3	11.2	9.2	9.5	9.4	43.3	40.7	42.0
Mexican Big Boll	14.5	14.3	14.4	11.6	10.7	11.2	36.0	34.0	35.0
Qualla	14.7	14.9	14.8	11.4	10.7	11.0	40.0	37.0	38.5
Rowden No. 2088	14.2	13.9	14.0	13.1	12.9	13.0	35.3	33.7	34.5
Startex No. 619	13.0	12.9	13.0	8.1	7.9	8.0	36.0	34.0	35.0
Stoneville No. 5	11.0	11.3	11.2	11.2	11.2	11.2	37.3	34.3	35.8
Triumph No. 44	12.3	12.1	12.2	10.6	10.2	10.4	34.7	33.0	33.8
Wilds No. 5	15.1	15.0	15.0	9.6	9.0	9.3	33.7	31.0	32.4
Average	12.9	12.9	12.9	10.3	10.3	10.3	36.6	34.5	35.5

[1] Average of 8 samples of 100 seeds each.
[2] Average of 3 determinations.
[3] Calculated from the mean seed index and the mean yield of lint (4 determinations).

10 pounds of lint, between the means of any two varieties of the 1936 crop can be considered highly significant. For similar significance, variety means of the 1937 crop must differ 4.49 watt-hours in the net energy required to gin 30 pounds of seed cotton and 4.12 watt-hours in net energy required to gin 10 pounds of lint.

There was considerable difference between the samples for the 2 years in the time and energy consumed. On the whole, the samples from the 1936 crop were ginned faster and required more net energy than those of 1937. A part of the difference in ginning requirements between the 2 years might be attributed to differences in seed cotton properties, but it is more probable that the greater part is due to variations in gin-stand operations, such as rate of feed and density of the seed roll. These variables were controlled by the gin-stand operator and were subject to error of judgment. The reasonableness of this explanation for differences in time and energy consumption between the 2 years is substantiated by the significant differences in ginning time and energy requirements between series within the same year (table 3). Differences between series are most logically assigned to variations in gin-stand operations. Since gin-stand operations would certainly vary more between years than between series of the same year, it is equally logical to assign a large part of the differences between years in ginning requirements to variations between the 2 years in ginning operation.

After having established that there are significant differences among varieties in the time and energy required to gin them, the next problem was to ascertain whether these differences could be explained by varietal differences in certain seed-cotton properties. Seed size (seed index), lint percentage, and amount of fuzz were the three properties selected for study (tables 4 and 5). The data were analyzed for covariance.

In order to combine the data for the 2 years into one analysis, it was necessary to omit the figures for Slick-seeded Acala since data for only 1 year were obtained for that variety. Too, since the number of tests made upon each variety were not the same for each year and since the seed-cotton properties were not measured for each ginning test separately, but for the variety as a whole, the covariance was determined on the variety means. Thus the test for significance falls upon the variety × year interaction (table 6).

TABLE 6.—*Mean squares for ginning and seed-cotton variables for 16 varieties grown at Stoneville, Miss., in 1936 and 1937*

Source of variance	Degrees of freedom	Independent variables			Dependent variables			
		Seed index	Lint percentage	Percentage of fuzz	Time—30 pounds of seed cotton	Time—10 pounds of lint	Energy—30 pounds of seed cotton	Energy—10 pounds of lint
Total	31	2.79	8.63	3.21	2.75	2.36	216.47	308.91
Years	1	36.98	26.46	5.36	27.75	10.81	5,000.00	6,418.44
Within years	30	1.65	8.04	3.14	1.92	2.08	57.02	105.26
Varieties [1]	15	3.12	15.57	6.14	3.27	3.76	102.86	193.07
Variety×year	15	.17	.50	.14	.57	.40	11.18	17.02

[1] Significant values of F for varieties $=\frac{\text{variety}}{\text{variety}\times\text{year}}=2.43$ at the 5-percent level; 3.56 at the 1-percent level.

Beta regression, and simple and multiple correlation coefficients, were calculated for the data as a whole and for the different sources of variance (table 7).

For the data as a whole the sizes of the simple correlation coefficients indicate that there is a significant tendency for an increase in the amount of fuzz upon the seed to be accompanied by an increase in the amount of time and energy consumed in ginning. Also, there is a definite tendency for the larger seeded cottons to require more time than the smaller seeded ones. However, the relationship between seed index and the amount of energy required is negative. This negative correlation coefficient is misleading. It results from the fact that the larger seeded crop of 1937 consumed less energy than the smaller seeded crop of 1936. But it has been pointed out previously that the difference between the 2 years in the amount of energy consumed is probably due to uncontrolled variations in gin-stand operations. This difference between the 2 years in energy consumed is so great that it has an enormous influence on the "over-all" correlation, and results in giving a false indication of relationships. If the variance due to years is eliminated from the data, leaving the "within years" variance, the relationship between seed index and energy consumed is positive; that is, within each year an increase in seed size is accompanied by an increase in the amount of energy consumed.

For the data as a whole, lint percentage has a significant influence only upon the amount of energy required to gin 10 pounds of lint. The correlation is negative. Cottons with high lint percentages require less energy to yield 10 pounds of ginned lint than cottons with low lint percentages.

Since there is little correlation among the seed-cotton properties, the beta coefficients for the "total" differ little from the simple correlation coefficients.

TABLE 7.—*Results of covariance analysis with simple and multiple correlation coefficients* [1] *and beta regression coefficients representing the relationship between the time and energy required to gin either 30 pounds of seed cotton or 10 pounds of lint, and seed index, lint percentage, and percentage of fuzz for 16 varieties grown at Stoneville, Miss., in 1936 and 1937*

Source of variance	Degrees of freedom	Simple correlation coefficients				Simple correlation coefficients among independent variables			Beta coefficients of independent variables			Multiple correlation coefficients	Standard error of estimate
		Dependent variable	Seed index	Lint percentage	Percentage of fuzz	Seed index and lint percentage	Seed index and percentage of fuzz	Lint percentage and percentage of fuzz	Seed index	Lint percentage	Percentage of fuzz		
Total	31	Time—30 pounds of seed cotton	0.584	0.339	0.448	0.062	0.049	−0.068	0.542	0.336	0.444	0.793	1.06
		Time—10 pounds of lint	.562	−.249	.501				.555	−.252	.456	.777	1.02
		Energy—30 pounds of seed cotton	−.362	−.280	.550				−.375	−.218	.553	.708	10.94
		Energy—10 pounds of lint	−.333	−.624	.466				−.319	−.577	.442	.819	10.61
Years	1												
Within years (varieties+varieties × years)	30	Time—30 pounds of seed cotton	.339	.204	.726	−.201	.273	.005	.206	.242	.668	.777	.92
		Time—10 pounds of lint	.445	−.422	.656				.203	−.384	.603	.806	.90
		Energy—30 pounds of seed cotton	.533	−.016	.712				.378	.057	.608	.797	4.81
		Energy—10 pounds of lint	.484	−.673	.494				.240	−.627	.432	.867	5.39
Varieties	15	Time—30 pounds of seed cotton	.369	.194	.827	−.185	.284	.012	.191	.219	.770	.866	.71
		Time—10 pounds of lint	.461	−.458	.721				.190	−.432	.673	.878	.73
		Energy—30 pounds of seed cotton	.601	−.044	.764				.423	.026	.643	.864	4.10
		Energy—10 pounds of lint	.508	−.689	.512				.262	−.647	.446	.899	4.82
Varieties × years	15	Time—30 pounds of seed cotton	.096	.411	−.498	−.060	.003	−.276	.450	.587	−.337	.673	.62
		Time—10 pounds of lint	.260	.133	−.455				.435	.290	−.377	.569	.58
		Energy—30 pounds of seed cotton	−.313	.454	−.111				−.062	.419	.005	.459	3.52
		Energy—10 pounds of lint	.152	−.448	.211				−.166	−.529	.065	.525	3.92

[1] Significant values of correlation coefficients:

Degrees of freedom:	At the 5 percent level		At the 1 percent level
		r	
30	0.349		0.449
29	.355		.436
14	.497		.623
		R	
28	.490		.573
27	.498		.582
12	.683		.773

The correlation coefficients of greatest interest, however, are those that represent the extent to which varietal differences in time and energy consumed are related to varietal differences in the three seed-cotton properties being considered. These coefficients were obtained for varieties taken as a whole and for varieties "within years." This latter represents the variance remaining in the data after that associated with yearly differences has been eliminated. The "within years" analysis is of special interest because of the very great differences between the 2 years in the amount of energy consumed.

The coefficients for varieties taken as a whole or "within years" are very similar and statements regarding one apply equally well to the other. Since there is some degree of correlation among the seed-cotton properties, the relative importance of each independent variable can best be ascertained by comparing the beta coefficients.

The simple correlation coefficients show that there is a significant tendency for varieties with large seeds and a high percentage of fuzz to require more time and energy to gin them than do varieties with smaller or less fuzzy seeds. Apparently large seeds or seeds with a heavy covering of fuzz are not discharged as rapidly from the gin-roll box during ginning as are smaller seeds or seeds with less fuzz, and consequently the larger or more fuzzy seeded varieties require more time and energy in the ginning process. The beta coefficients show that the percentage of fuzz is the more important of these two seed-cotton properties, especially in determining the amount of time consumed—seed size apparently having more influence upon the amount of energy consumed than upon the time required to gin unit weights of seed cotton or to yield unit weights of ginned lint.

According to the simple correlation coefficients, there is little or no relation between lint percentage and the time and energy required to gin 30 pounds of seed cotton, but there is a significant relation between lint percentage and the time and energy required to gin enough seed cotton to produce 10 pounds of lint. This relationship is negative, the varieties with a high lint percentage requiring less time and energy to yield 10 pounds of lint than do those with a low lint percentage. The beta coefficients indicate that lint percentage is more important than seed size but less important than the amount of fuzz in its effect upon the amount of time consumed in ginning either 30 pounds of seed cotton (positive effect) or 10 pounds of lint; that it has practically no influence upon the amount of energy required to gin 30 pounds of seed cotton; but that it is the most important of all three factors in its effect upon the amount of energy required to gin 10 pounds of lint.

Correlation coefficients for the variety $\times$ year interactions are on the whole relatively insignificant, indicating that tendencies which certain varieties may have shown to require relatively more energy or time in 1 year than in the other cannot be related to differences in any of the seed-cotton properties. It is true that a few of the correlation coefficients indicate some degree of relationship, but it must be borne in mind that technical variations which were not completely controlled may confuse the true relationships.

The strength of fiber attachment to the seed was determined for the 1936 crop, and significant differences were found between varieties in the force required to detach fibers from the seed. [7] But differences in

[7] SMITH, W. S., and PEARSON, N. L. A METHOD OF MEASURING THE STRENGTH OF ATTACHMENT OF COTTON FIBERS TO THE SEED AND SOME RESULTS OF ITS APPLICATION. United States Agr. Market Serv., and Bur. Plant Indus., 22 pp., illus. 1941. [Processed.]

mean strength of fiber attachment to the seed were found not to be related to corresponding differences in the amount of energy required to gin these particular cottons. However, in considering the question of strength of fiber attachment in relation to ginning, it is necessary to take into account the total number of fibers which must be detached, and the total force which would be required to detach all of them. This total force was calculated for each variety by multiplying the calculated number of fibers in 30 pounds of seed cotton by the mean strength of fiber attachment. The multiple correlation coefficients and the beta coefficients were then calculated to show the combined and individual effects of seed index, percentage of fuzz, and total force required to detach the fibers upon the energy required to gin the cottons (table 8). The R value obtained was significant, but the beta coefficients indicated that the total force required to detach the fibers had, in the case of these particular varieties, no effect upon the energy consumed. For this reason, the strength of fiber attachment was not measured for the 1937 crop.

The first and second pickings of the 1937 crop were ginned separately and showed significant differences in ginning behavior (table 3). The second picking required significantly more time and energy than the first. It contained more trash, which would account, in part at least, for the greater amount of time and energy consumed. It also had a lower lint percentage (table 5), which in addition would bring about an increase in the time and energy required to gin 10 pounds of lint.

TABLE 8.—*Multiple correlation and beta regression coefficients showing relation between the energy required to gin 30 pounds of seed cotton and the seed index, percentage of fuzz, and mean strength of fiber attachment × number of fibers in 30 pounds of seed cotton, for 16 varieties grown in 1936*

Dependent variable	R [1]	Beta cofficients of independent variables		
		Seed index	Percentage of fuzz by weight	Mean strength of fiber attachment × number of fibers
Energy required to gin 30 pounds of seed cotton, 1936	0.903	+0.572	0.531	0.071

[1] Significant values of R: at the 5-percent level=0.683; at the 1-percent level=0.773.

SUMMARY

Tests were conducted to ascertain whether certain American upland cottons differed in respect to the time and energy required for ginning and, if so, to ascertain whether variations in certain seed-cotton properties were responsible for these differences.

Plantings of 16 varieties in both 1936 and 1937, and a seventeenth in 1937 only, provided seed cotton for 184 test lots of 30 pounds each—3 for each variety in 1936 and 8 for each variety in 1937.

Varieties were found to differ significantly in the time and net energy required to gin either 30 pounds of seed cotton or enough seed cotton to produce 10 pounds of lint.

The larger and more fuzzy-seeded cottons required more time and energy to gin than the smaller and less fuzzy-seeded varieties. Apparently the large and fuzzy seeds are not discharged from the roll box during ginning as rapidly as smaller and less fuzzy seeds.

Varieties with a high lint percentage required less time and energy to gin 10 pounds of lint than varieties with low lint percentage. Lint percentage, however, had little effect upon the energy required to gin 30 pounds of seed cotton, but did affect the amount of time consumed, an increase in lint percentage tending to be accompanied by an increase in the amount of time required.

The order of influence of the three seed-cotton properties follows. Time required to gin 30 pounds of seed cotton or 10 pounds of lint: (1) Percentage of fuzz, (2) lint percentage, and (3) seed size. Net energy required to gin 30 pounds of seed cotton: (1) Amount of fuzz, (2) seed size, and (3) lint percentage. Net energy required to gin 10 pounds of lint: (1) Lint percentage, (2) amount of fuzz, and (3) seed size.

Strength of fiber attachment to the seed had no effect upon the energy required to gin these particular cottons.

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FUNGICIDAL TESTS ON BLUE MOLD (PERONOSPORA TABACINA) OF TOBACCO[1]

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INTRODUCTION

In 1938 Clayton et al. (*1*, *2*)[2] reported a copper oxide-cottonseed oil spray treatment for the control of the blue mold (*Peronospora tabacina* Adam) disease of tobacco (*Nicotiana tabacum* L.). It was demonstrated that this spray treatment reduced disease development to such a degree that even under severe conditions measurable damage was prevented; i. e., there was no appreciable retardation of plant growth or reduction in the number of plants produced. Since 1938 these findings have been repeatedly verified, and the treatment has been used extensively by tobacco growers. The problem of spray control for blue mold, however, has been of great interest not only because of the practical control afforded but also because of the ques tions raised as to the nature of the protection that the spray provides.

As reported previously, bordeaux mixture is not effective against blue mold, nor is copper oxide effective when used alone. However, copper oxide when combined with cottonseed oil gives good control of the disease. The oil, as will be shown later, contributes the chief disease-control value of the mixture. While mineral oils are used extensively in present-day spray practice as insecticides, and to a limited degree with copper fungicides as spreaders and stickers, the vegetable or glyceride oils have been used very little. They are somewhat more expensive than the mineral oils, and not so readily emulsified. However, the glyceride oils have been shown to possess both insecticidal and fungicidal value; they are essentially mixtures of the glycerides of various fatty acids, and Siegler and Popenoe (*14*) have demonstrated that caproic and lauric acids are efficient contact insecticides. Martin and Salmon (*12*) in 1933 reported that a number of vegetable oils were fungicidal against the hop powdery mildew. The first objective of the present investigation, consequently, was to discover more of the fungicidal possibilities of the vegetable oils. Certain animal and mineral oils, as well as nonglyceride oils of vegetable origin, were included in some experiments for purposes of comparison. Since the word "fungicidal" has had broad as well as restricted usage, it is well to mention here that in the following discussion the term "fungicidal" refers to either destruction of the fungus or inhibition of fungal growth. No attempt has been made to distinguish between fungicidal and fungistatic action. Inhibition of

[1] Received for publication May 14, 1942. Cooperative Investigations of the Division of Tobacco Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Georgia Coastal Plain Experiment Station, the North Carolina Department of Agriculture, and the Agricultural Experiment Stations of North Carolina, South Carolina, and Maryland.

[2] Italic numbers in parentheses refer to Literature Cited, p. 275.

growth was soon followed by the death of the tobacco blue mold (downy mildew) fungus.

METHODS OF STUDY

Blue mold has proved a very convenient disease with which to conduct fungicidal investigations. It attacks the plants as seedlings, and greenhouse conditions during the late fall, winter, and early spring months are most favorable for its development. The usual procedure has been to sow tobacco seed in 6-inch pots, and thin to a uniform stand. After the pots had received 3 to 4 applications of spray, they were inoculated with a spore suspension and placed under conditions favorable for disease development. Sprays were applied twice weekly for a total of 7 to 11 treatments. It was possible to complete from 8 to 10 separate spray experiments each winter. Blue mold affects the crop in several ways, and spraying results have been measured in terms of leaf-disease and plant-survival counts. The greenhouse work has been checked and supplemented by extensive tests conducted in plant beds at the Georgia Coastal Plain Experiment Station, Tifton, Ga.; the Pee Dee Experiment Station, Florence, S. C.; tobacco branch stations at McCullers and Oxford, N. C.; the Arlington Experiment Farm, Arlington, Va.; and the branch station of the Maryland Agricultural Experiment Station at Upper Marlboro, Md.

FUNGICIDAL VALUE OF VARIOUS SUBSTANCES

OILS

Tests were made with a wide variety of oils, and the data in table 1 will serve to show the great range in effectiveness of different oils. All the oils in this experiment were used at the 1-percent level excepting olive, pine, and sperm oils, which were used at the ½-percent level because at 1 percent they caused leaf injury. The oils were emulsified with Vatsol O. T. C.[3] at the rate of 1 pound per gallon.

TABLE 1.—*Fungicidal value of various oils*

Kind of oil	Diseased leaves[1]				
	Plot 1	Plot 2	Plot 3	Plot 4	Mean[2]
	Number	*Number*	*Number*	*Number*	*Number*
Beef suet	16	17	35	30	24.50
Castor	21	17	30	34	25.50
Coconut	15	30	27	19	22.75
Cod-liver	0	3	4	8	3.75*
Corn	0	3	2	9	3.50*
Cottonseed	1	10	3	13	6.75*
Eucalyptus	14	13	26	39	23.00
Linseed	0	0	3	3	1.50*
Olive	21	20	27	38	26.50
Palm	12	15	27	37	22.75
Paraffin	25	30	31	36	30.50
Peanut	3	1	12	17	8.25*
Pine	29	25	33	37	31.00
Rapeseed	1	4	5	16	6.50*
Sesame	9	1	16	7	8.25*
Soybean	0	3	0	2	1.25*
Sperm	8	12	3	3	6.50*
Tung	1	1	4	4	2.50*
Check	22	23	27	26	24.50
Check	21	20	31	39	27.75
Average					26.13

[1] Total number of leaves exposed in each plot, 45.
[2] Values significantly lower than the average of the 2 checks (8 plots) at the 1-percent level are indicated by an asterisk (*).

[3] This detergent was reported by the manufacturer to have as its active ingredient 10 percent of sodium dioctyl sulfosuccinate.

As the data in table 1 indicate, tung, linseed, soybean, rapeseed, sesame, and cottonseed oils all gave good control of blue mold. On the other hand, beef suet, castor, coconut, eucalyptus, olive, paraffin, palm, and pine oils gave no significant reduction in the amount of disease. Paraffin oil in some experiments actually increased the amount of disease injury. Other oils tested, but not included in this experiment, were chaulmoogra, cade, copaiba, cedarwood, clove, lemon, mirbane, mustard, origanum, peach-kernel, sassafras, turtle, and resin. Most of these had little or no fungicidal value. Since oils of the fungicidally active group were all of the glyceride type, it is of interest to compare their fatty acid content with the fatty acid content of the fungicidally inactive group of glyceride oils.

The data in table 2 represent average values as reported by Jamieson (*8*) and Lewkowitsch (*9*), and it may be noted first that both fungicidal

TABLE 2.—*Fatty acid content of fungicidal and nonfungicidal glyceride oils*

Group and kind of oil	Predominant fatty acid									
	Oleic	Linoleic	Linolenic	Eleostearic	Ricinoleic	Palmitic	Lauric	Chaulmoogric	Stearic	Myristic
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Fungicidal:										
Cottonseed	30	45								
Corn	43	50								
Linseed	11	39	36							
Peanut	55	23								
Soybean	45	35								
Tung	13			73						
Nonfungicidal:										
Beef tallow	(1)						(1)		(1)	
Castor					80					
Coconut							44			24
Chaulmoogra								(1)		
Olive	85									
Palm	43					36				

[1] Present in appreciable amount but exact figure not available.

and nonfungicidal oils contain much oleic acid, so that fungicidal activity cannot be easily correlated with the occurrence of this fatty acid. Linoleic acid, however, occurs in large amounts in most of the fungicidal oils, but not to any extent in the nonfungicidal oils. There are strong indications also that linolenic acid (linseed oil) and eleostearic acid (tung oil) are associated with positive fungicidal activity; as is also licanic acid (oiticica oil), which is closely related to eleostearic acid. These make up a compact group of fatty acids having 18 carbon atoms and 30 to 32 hydrogen atoms. Ricinoleic, palmitic, lauric, chaulmoogric, stearic, and myristic acids were apparently not active fungicides.

COPPER AND SULFUR COMPOUNDS

Extensive tests were made on all copper compounds obtainable. The results were not of sufficient value to be given in detail. Mixtures such as bordeaux and copper-soap sprays delayed the development of the disease sufficiently to give fair control under favorable conditions. However, with severe outbreaks, when protection is most desired, they have failed. Similarly, partial but unsatisfactory control has been obtained with sulfur sprays, such as Sulfocide and Yarwood's

resin-lime sulfur, which also had the disadvantage of retarding growth. The most serious disadvantage of the use of copper or sulfur compounds alone is that a greater proportion of sprayed plants than of unsprayed plants may die after being transplanted.

COPPER-OIL SPRAYS

The comparative effectiveness against blue mold of sprays containing oil alone, copper alone, and the two in combination is indicated by the data in table 3, which give the results of a representative greenhouse experiment.

TABLE 3.—*Fungicidal value of copper and oil sprays alone and in combination*

Copper and oil sprays	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Mean [2]
DISEASED LEAVES [1]							
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Copper oxide plus cottonseed oil	0	2	2	6	3	1	2.3
Bordeaux plus cottonseed oil	5	4	3	1	1	6	3.3
Cottonseed oil alone	4	4	14	17	10	8	9.5
Copper oxide alone	31	29	22	28	18	22	25.0
Bordeaux alone	35	41	36	42	30	32	36.0
Check	168	149	147	149	157	148	153.0
PLANTS KILLED [3] [4]							
Copper oxide plus cottonseed oil	5	14	7	15	8	7	9.3
Bordeaux plus cottonseed oil	17	10	10	8	16	16	12.8
Cottonseed oil alone	15	16	21	20	21	19	18.7
Copper oxide alone	26	34	32	29	34	29	30.7
Bordeaux alone	33	34	39	36	29	37	34.7
Check	36	37	38	36	36	38	36.8

[1] There were approximately 175 leaves per plot.
[2] Significant difference between any 2 means at 5-percent level, 6.1; at 1-percent level, 8.3.
[3] There were approximately 43 plants per plot; plant data were taken 3 weeks after the leaf data.
[4] Significant difference between any 2 means at 5-percent level, 4.0; at 1-percent level, 5.5.

The sprays were applied twice weekly, beginning January 1, 1938, and by January 20 the untreated check plants were practically defoliated. Leaf counts were made at this time, and data on plants killed were taken on February 16. Ten sprays in all were applied. Considering first the data on diseased leaves, the difference between copper oxide-oil and bordeaux-oil sprays was insignificant at the 5-percent level, and on the same basis both copper-oil sprays were significantly better than oil alone. Oil alone was much superior to either copper oxide or bordeaux alone. Passing now to the end results measured in terms of plants killed, it is to be noted that neither copper oxide nor bordeaux alone appreciably reduced plant mortality, and the maximum protection was provided by the two copper-oil combinations; the oil alone was better than either copper compound, but not equal to the copper-oil combinations. It is readily possible to show by computation that the protection provided by the combination of copper and oil is significantly greater than the sum of the protection provided by each separately. The fact that the oil portion of the copper-oil spray is more important as a fungicide than the copper, in blue mold control, is emphasized because many have erroneously regarded the copper as the active ingredient. The relative fungicidal merits of oil and copper are illustrated by figure 1.

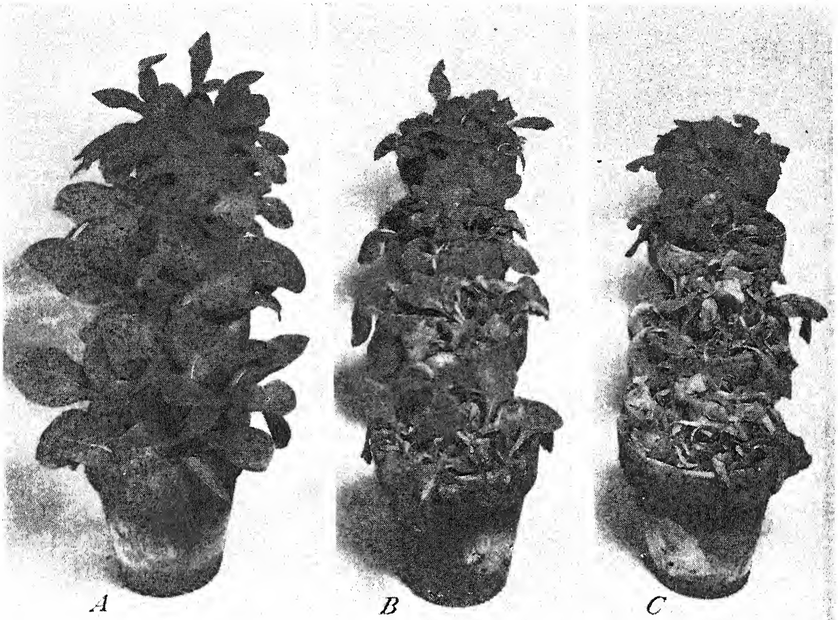

FIGURE 1.—Tobacco plants sprayed twice weekly with (*A*) 1-percent cottonseed oil and (*B*) copper oxide (Cuprocide), 1 pound per 100 gallons; *C*, untreated check. Note that the oil spray gave marked blue mold control, while the copper spray was ineffective.

The data in table 3 are from a greenhouse experiment in which disease was very severe, as indicated by the high plant mortality in the checks and the extent of disease that developed in lots receiving the best treatment. In table 4 data for a plant-bed experiment indicate that effective control can be secured with various copper compounds in addition to cuprous oxide, provided they are combined with the oil. These data also illustrate the very much higher degree of protection that can be obtained in the usual plant-bed experiment.

The results in table 4 show that all copper-oil mixtures gave effective plant-stand protection. Many leaves in the sprayed plots showed infections, but these remained small, and little or no leaf tissue was

TABLE 4.—*Fungicidal value of different copper-oil mixtures*

Spray mixture	Leaf area killed [1]				Plants killed [2]			
	Plot			Mean	Plot			Mean
	1	2	3		1	2	3	
	Percent	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Number*
Copper tartrate plus cottonseed oil	4.70	0.55	3.43	2.89	0	0	0	0
Copper oxalate plus cottonseed oil	0	0	0	0	0	0	0	0
Copper oxide plus cottonseed oil	2.66	0	0	.89	0	0	0	0
Untreated check	93.39	93.17	97.08	94.55	30	24	35	29.

[1] Based on a 15-leaf sample taken at random in each plot.
[2] The number of plants per plot was 42 to 45.

killed. Data in table 4 are from an out-of-door plant-bed experiment, those in table 3 from a greenhouse test. As usual with the less severe plant-bed conditions, the sprays gave more perfect disease control. All readily available copper compounds have been tested in combination with oil, and particularly good results have been obtained with the oxide, resinate, oxalate, tartrate, and oxychloride. Tests with various cuprous oxides have shown slightly better results when the very finely divided yellow oxide obtained by the Raleigh (*13*) procedure was used. The ordinary commercial red and orange oxides, however, have given satisfactory results, and they are readily obtainable. In general, the results with cuprous oxide were slightly superior to those secured with other copper salts, with the possible exception of the oxalate. Earlier it was shown (table 1) that a number of vegetable oils were fungicidal, and some, e. g., tung, soybean, and corn, were slightly superior to cottonseed. Extensive tests have been conducted with these in combination with copper oxide, but none of the combinations were superior to the cupric oxide-cottonseed oil. Cottonseed oil has the further advantage of being readily available throughout the entire Southeast. However, the combination of copper oxide and soybean oil was carefully tested and proved entirely satisfactory.

As shown in table 1, certain oils used alone gave good control of mold, and such oils as tung and soybean were definitely superior to cottonseed. If copper is omitted, tobacco plants will tolerate a 2-percent oil emulsion, and careful tests were made to compare the copper-oil combination with strong oil emulsions. Results of these tests are shown in table 5.

TABLE 5.—*Fungicidal value of strong oil emulsions*

Spray	Diseased leaves [1]					
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Mean [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
CuO plus 1 percent cottonseed oil	5	3	2	4	4	3.6
Oiticica oil, 2 percent	2	16	9	13	4	8.8
Tung oil, 2 percent	3	3	4	4	1	3.0
Soybean oil, 2 percent	7	13	17	15	24	15.2
Cottonseed oil, 2 percent	15	23	17	24	30	21.8
Linseed oil, 2 percent	0	0	4	4	5	2.6
Check	166	168	170	173	173	170.0
Do	175	169	168	170	173	171.0

[1] There were approximately 175 leaves per plot.
[2] Significant difference between any 2 means at 5-percent level, 3.95; at 1-percent level, 5.3.

The data in table 5 are representative of a large number of tests conducted in the greenhouse. On the whole, the best results were secured with 2-percent tung oil, and this mixture together with several others was used in a number of plant-bed experiments in 1939. In these tests, the 2-percent tung oil gave good mold control but was not quite equal to the regular mixture. Other experiments had already shown that, although under some conditions tung oil and linseed oil were superior to cottonseed oil if used alone, when combined with the copper all were equally effective. Therefore, it was concluded that, while a 2-percent oil spray could be used for blue mold control, the copper-oil combination was preferable. In general it has appeared that (1) the copper-oil combination reduced the number and size of

lesions to a minimum; (2) the oil alone greatly reduced the size of lesions but permitted a considerable number of infections; and (3) the copper alone temporarily reduced the number of infections, but this merely delayed disease development for a short time until the amount of infection had increased. These several effects are illustrated in figure 2.

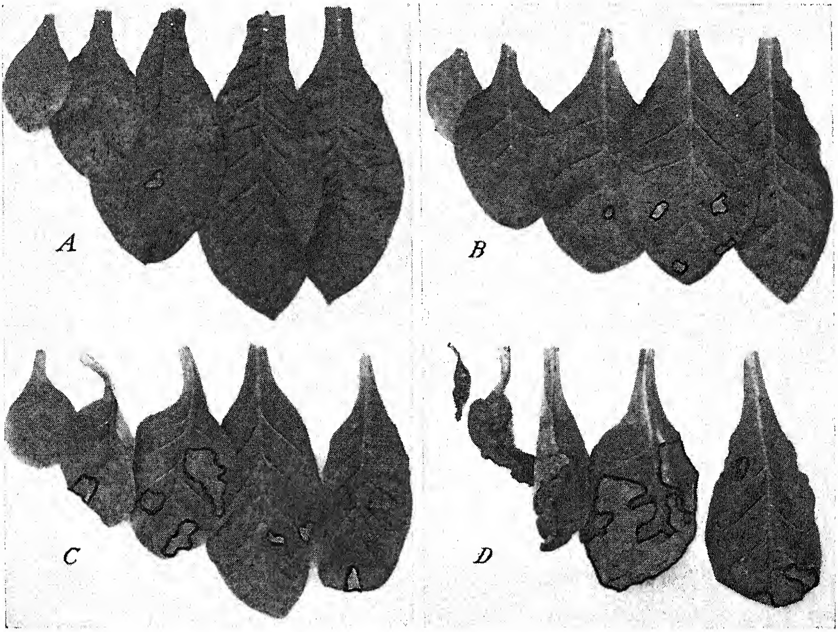

FIGURE 2.—Results of spraying tobacco plants with (*A*) regular copper oxide-oil mixture (one lesion in center of third leaf from left outlined in black), (*B*) 2-percent cottonseed oil (five lesions on two leaves; small lesions indicate inactive infections), (*C*) cuprous oxide, 2 pounds per 100 gallons (seven lesions on four leaves; infections spreading rapidly). *D*, Untreated check; left to right, first three leaves completely invaded by fungus, fourth leaf severely diseased, and fifth leaf slightly so.

NUTRIENT SALTS IN COMBINATION WITH OIL SPRAYS

As will be brought out later, vegetable oils, such as cottonseed oil, at the concentrations used by the writers were not toxic to the blue mold fungus. Control of the disease apparently resulted from the development of a marked resistance by the plant that restricted the spread of the fungal hyphae after the infection had occurred. The oil penetrated the leaf tissues freely, and numerous globules could be seen in the intercellular spaces. It has seemed possible that the value of the oil sprays might be increased by the addition of nutrient salts. Thus, Henderson (*6*) found that in sand culture nitrogen and potassium both had marked influence on blue mold development. Preliminary experiments showed that 0.5 percent of sodium nitrate ($NaNO_3$) and urea, and 1.0 percent of potassium sulfate (K_2SO_4) and extract of cottonseed meal could be added to the oil sprays without injury to the plants.

Repeated experiments were made with cottonseed, tung, and linseed oils in combination with the different nutrients. In no case was the combination superior to the oil emulsion alone, and the cottonseed extract actually destroyed the fungicidal value of each oil to which it was added. As the addition of cottonseed meal extract did not cause any visible change in the mixtures, the reason for the unfavorable effect was not clear.

MISCELLANEOUS FUNGICIDES

The possibility of substituting silver nitrate for the copper oxide was investigated, and such combinations were tested both in the greenhouse and plant bed. The silver nitrate-oil mixtures had distinct fungicidal value but were not quite equal to the copper oxide-oil mixtures. Paradichlorobenzene, benzene, pentachloroethane, and xylene, all of which have value as vapor treatments, were tested in combination with oil alone, and were added to the copper oxide-oil mixture. They showed a slight value in some tests, but after careful consideration were finally discarded. Malachite green, recommended in a treatment for a similar disease of hops, showed no value against tobacco blue mold.

A number of the organic mercurials were also tested without obtaining satisfactory results.

MISCELLANEOUS ORGANIC COMPOUNDS

Early in 1939 a broad consideration of organic materials was undertaken. Each compound selected was tested with oil and also, in most cases, without oil. Alcohol, acetone, and benzene were used as solvents. A few insoluble materials were used as suspensions, with a suitable wetting and dispersing agent. Tests with each compound were continued until definite proof was obtained that it did not give adequate disease control at a phytocidal level. To date, 122 organic compounds have been tested, and all that showed fungicidal promise were included in repeated experiments.

It would be impossible to present a detailed report of these experiments, since the volume of data is necessarily large. A considerable number of the compounds tested possessed distinct fungicidal value; e. g., tetramethylthiuram disulfide, 5–hydroxyl–1, 3-dimethyl benzene, *p*-toluenesulfonyl chloride, *o*-cresyl *p*-toluenesulfonate, ferric dimethyldithiocarbamate, and salicylic acid. This last compound was first tested against blue mold at the Georgia Coastal Plain Experiment Station, Tifton, Ga., in the spring of 1939. Subsequent experiments have pointed more and more to the salicylates in general as the most promising of all the compounds studied. An indication of the comparative merits of certain salicylates and copper oxide is supplied by the data in table 6, which are from a greenhouse experiment.

The plants from which the data in table 6 were taken received 7 spray applications, the last on January 6. Conditions were ideal for the development of blue mold during the entire period of the experiment. Diseased leaves were removed as counted; hence the final column in table 6 gives the total percentage of diseased leaves. The best spray mixture was zinc salicylate, ¼ pound per 100 gallons;

TABLE 6.—*Fungicidal value of the salicylates*

Spray mixture[1] and concentration per 100 gallons	Leaves[2] diseased on—			Total
	Jan. 15	Jan. 23	Jan. 31	
	Percent	*Percent*	*Percent*	*Percent*
Zinc salicylate, ¼ pound	4.11	5.53	9.46	19.10
Zinc salicylate, ⅛ pound	7.14	13.75	20.00	40.89
Phenyl salicylate, ⅛ pound	5.53	12.32	16.96	34.81
Phenyl salicylate, 1/16 pound	10.71	16.25	19.40	46.42
Salicylamide, ¼ pound	10.19	15.17	17.86	43.22
Salicylamide, ⅛ pound	11.79	15.00	15.89	42.68
Salicylic acid, ⅛ pound	7.50	15.00	17.50	40.00
Cuprocide 54 Y 1½ pound	14.28	19.28	21.78	55.34
Untreated check	100.00	100.00	100.00	100.00

[1] Each with 1 percent cottonseed oil.
[2] There were 140 leaves per plot. Each value is the mean of 4 plots.

the plants receiving this treatment showed noticeably less disease throughout the experiment (fig. 3). Salicylamide, which is said to be the active ingredient of Shirlan A. G., used as a spray against tomato mold in England, was not equal to zinc salicylate. Among the various salicylates tested, excellent results were obtained with methyl salicylate under cool conditions; at higher temperatures, however, this material was not effective. On the other hand, benzyl salicylate, which is also a liquid, was highly effective under a wide variety of conditions. It might appear that methyl salicylate was more easily lost by vaporization, but the boiling point of methyl salicylate is 220°–224° C. and that of benzyl salicylate 208°, so this explanation would seem unsatisfactory.

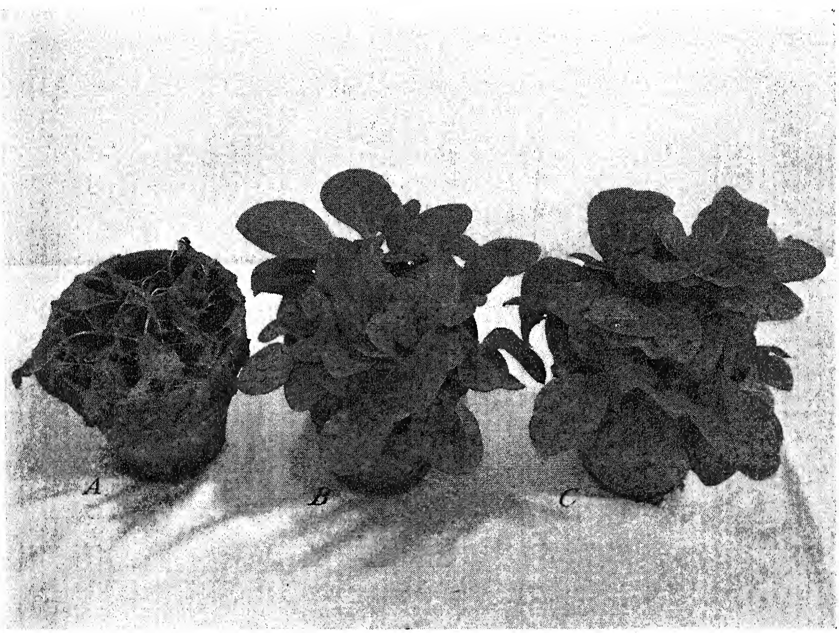

FIGURE 3.—*A*, untreated check; *B*, control of blue mold with copper oxide-oil mixture; *C*, control of blue mold with zinc salicylate-oil mixture.

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The spring of 1941 was not favorable for the development of blue mold, and the results obtained in plant-bed experiments were not decisive. Nevertheless, salicylates gave promising results. At Florence, S. C., and later at Arlington, Va., bismuth salicylate without oil showed up exceptionally well. The results of an experiment in which some of the better salicylates were used are given in table 7.

TABLE 7.—*Blue mold control in the plant bed with the salicylates*

Spray and concentration per 100 gallons	Diseased leaves							
	5 days after final spray				20 days after final spray			
	Plot 1	Plot 2	Plot 3	Mean [1]	Plot 1	Plot 2	Plot 3	Mean [2]
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Regular copper oxide-oil	7	4	3	4.67	28	28	32	29.33
Benzyl salicylate, ¼ pound, plus 1 gallon of oil	0	0	0	0	5	5	9	6.33
Zinc salicylate, ¼ pound, plus 1 gallon of oil	4	2	0	2.0	9	12	8	9.67
Salicylic acid, ½ pound, plus 1 gallon of oil	12	8	6	8.67	9	11	16	12.00
Bismuth salicylate,[3] 1½ pound, no oil	3	1	2	2.0	4	3	7	4.67
Untreated check	42	55	40	45.67	3	5	5	4.33

[1] Significant difference between any 2 means at 5-percent level, 6.66; at 1-percent level, 9.47.
[2] Significant difference between any 2 means at 5-percent level, 3.45; at 1-percent level, 4.91.
[3] There are two bismuth salicylates; the one used in this experiment was the basic bismuth salicylate, 64 percent Bi_2O_3.

The data in table 7 show that benzyl salicylate at a dilution of 1 to 3,200 (¼ pound per 100 gallons) plus a 1-percent oil gave perfect disease control at the time of the first disease count, which was made 5 days after the final spray application. However, all the mixtures used had given good control at the time. The last disease count was made 20 days after spraying ceased, and by this time the unsprayed check had recovered and so showed very little active disease. At this time the copper-sprayed plots were noticeably the most severely diseased and showed a marked increase of infection. Certain salicylate-sprayed plots, on the other hand, were still quite free of blue mold. This tendency of the salicylates to provide spray protection over a much longer period than the copper compounds has been observed many times.

EMULSIFIERS FOR COPPER-OIL AND SALICYLATE-OIL MIXTURES

While the vegetable oils do not emulsify as easily as mineral oils, stable preparations can readily be prepared with a wide variety of emulsifiers. The addition of copper or zinc compounds, however, greatly reduces the number of adequate emulsifiers, and still further restriction is imposed by the requirement that emulsification must be possible with waters of varying hardness. Thus, tests throughout the Southeast have shown that waters of the Coastal Plain area are quite soft, having less than 100 p. p. m. of hardness, whereas in the Piedmont belt some well waters test up to 250 p. p. m. of calcium carbonate ($CaCO_3$).

Various common soaps were tested as emulsifiers and found unsatisfactory. This was true also of sulfated vegetable oils, calcium caseinate, bentonite, kaolin, sulfite lye, gelatin, triethanolamine oleate, monoamylamine oleate, diglycol laurate, glycerol mono-

stearate, diglycol stearate, ammonium stearate, ammonium linoleate, aminostearin, and glycerol monoricinoleate. More promising results were obtained with certain members of a group of complex new synthetic detergents that have been developed recently for use particularly in the textile trade.

The development of the salicylate-oil sprays required a resurvey of the emulsifier situation. Vatsol O. T. C., which was excellent with the copper-oil mixture, could not be used in the salicylate-oil combinations. On the other hand, B1956 [4] proved very satisfactory with zinc and benzyl salicylates. An additional and reasonably priced emulsifier that was compatible with the benzyl salicylate-oil mixture, but not with zinc, was Twitchell Base 400H.[5] It was easily possible with the salicylates to make all-in-one mixtures that merely required dilution. Zinc salicylate dissolves in alcohol, acetone, or benzene, and these solutions in turn are readily soluble in oil. Benzyl salicylate, of course, is directly oil-soluble, and the B1956 emulsifier was oil-miscible. With these in an all-in-one mixture, the procedure to prepare a batch of spray was very simple. First, the proper amount of stock was measured out, a little water was added, and the mixture was then forced through a spray nozzle, which produced effective emulsification. The emulsion was then diluted to the proper volume.

TOXICITY OF OILS, CUPROUS OXIDE, AND BISMUTH SALICYLATE TO SPORES OF PERONOSPORA TABACINA

McCallan and Wilcoxon (*11*), Horsfall et al. (*7*), and other workers have found the spore-germination test a valuable aid in their efforts to develop an adequate laboratory method for the evaluation of copper fungicides. It is obvious that if other spray materials could be quickly tested in the laboratory and even approximate results obtained, much laborious greenhouse and field work would be saved. The writers made germination tests with cottonseed oil, palm oil, and cuprous oxide. Spores on a slide treated with 1-percent oil, or in actual drops of the oil emulsions, germinated normally. Although cottonseed oil controls blue mold and palm oil does not (table 1), the germination of *Peronospora tabacina* spores was higher with cottonseed than with palm oil emulsion or with tap water. Cuprous oxide, at the concentration used for spraying, inhibited all spore germination. Thus, the spore-germination tests would lead to the conclusion that cuprous oxide is a very effective spray and that cottonseed oil is completely ineffective. Figure 1 and table 3 show that this is not the case and consequently that the fungicidal value of the oils cannot be explained on the usual basis of protective leaf coatings that destroy the disease-producing spores before penetration and infection can occur.

Spore-germination experiments were also conducted to measure the comparative toxicity of cuprous oxide and bismuth salicylate. The former (table 3) was ineffective as a blue mold spray unless combined with oil, while the latter (table 7) used alone gave good control. In conducting the spore toxicity tests the initial concentration of each material was the same as had previously been used in the sprays, and a sufficient series of dilutions was made to cover the entire range

[4] A phthalic anhydride glycerol alkyl resin.
[5] Reported by the manufacturer to be a sulfonated mineral oil.

from complete inhibition of spore germination to complete absence of measurable toxicity. The lowest concentration of cuprous oxide definitely toxic to the spores was 0.0058 pound per 100 gallons; that of bismuth salicylate, 0.094 pound per 100 gallons. On this basis, the copper compound was about 16 times the more toxic. The lowest concentration of cuprous oxide that completely inhibited all spore germination was 0.094 pound per 100 gallons; that of bismuth salicylate, 0.375 pound per 100 gallons. On this basis, the copper compound was 4 times the more toxic.

There were other interesting differences between these two materials. Under the microscope the cuprous oxide particles appear as irregular solid masses. The bismuth salicylate particles are also very small and, furthermore, each particle is a group of slender crystals that tend gradually to separate. With the aid of Vatsol O. T. C., the bismuth makes an excellent suspension. The effect of cuprous oxide and bismuth salicylate on the fungus spores also is different. Cuprous oxide in toxic amounts causes marked cell plasmolysis, whereas bismuth salicylate, even in concentrations that inhibit germination, produces no plasmolysis.

DISCUSSION

For many years, copper fungicides have been relied upon almost exclusively for the control of fungus diseases of the herbaceous crops. The present war situation has made it quite apparent that fungicides requiring little or no copper would be most desirable. Therefore, the results of the study reported here, which deal entirely with the control of the blue mold disease of tobacco, may have a broader significance. Furthermore, it is of interest to note that the vegetable oils, and particularly such materials as bismuth and benzyl salicylate, offer promise not merely as substitutes for copper but as materials that may actually be superior to copper compounds in convenience and in disease control.

Again, the usually accepted explanation of spray protection against diseases is that, by coating the exposed leaf or fruit surface with a fungicide, the germinating spores are destroyed and infection is prevented. Anyone familiar with plant-bed culture of tobacco can appreciate that in the latter stages of growth, when the plants form a solid mass of foliage with many leaves close to the ground, it would be impossible to coat all leaf surfaces with a fungicide. In the spraying work here reported this has not been attempted, the effort being (1) to coat exposed surfaces and (2) to apply the spray three or four times before the disease developed. It has been shown that the oils differ greatly in fungicidal value against blue mold and that these differences have been associated with the glyceride content. Oils containing appreciable amounts of linoleic, eleostearic, or licanic glycerides had strong fungicidal value, while oils containing lauric, myristic, palmitic, chaulmoogric, oleic, and stearic glycerides were inactive. Indicating different reactions with different diseases, Fajans and Martin (*4*) reported that cuprous oxide plus mineral oil was superior to cuprous oxide plus cottonseed oil against late blight (*Phytophthora infestans* (Mont.) D By.) of potato. This is the direct reverse of the results the writers have obtained with blue mold.

It is of interest to consider just how such a material as cottonseed oil functions in blue mold control. The oil acts as a sticker for the

copper, but ineffective oils are just as good stickers. The oil-sprayed leaves dry earlier in the morning, and also sooner after rains, than the leaves of unsprayed plants, but here again ineffective oils also favor quick drying of leaves. Finally, spores of the blue mold fungus germinate as readily in 1-percent cottonseed oil as in clear water. The oil-sprayed leaves become a darker green after three or four applications (fig. 4), and microscopic examination of the tissues at this stage shows numerous oil globules in the intercellular spaces. When such leaves become infected, the mycelium spreads for a short time, then ceases to grow. Ultimately only a small area is killed, instead of an entire leaf. The visible reaction of the invaded oil-treated tissues is the same as the reaction of leaf tissue possessing genetic resistance (such as the *Nicotiana tabacum* × *N. debneyi* tetraploid). Thus, it appears that the oil-treated leaves are resistant to blue mold, and that in some manner certain oils promote the development of this resistance.

Since the presence of the oil inside the leaf has the effect just described, it might be thought that similar resistance could be built up with copper, but this does not appear to be the case. Thus, De Ong (*3*) has shown that, with a copper compound dissolved in an oil, considerable amounts of the copper could be recovered from the leaf tissues, whereas the usual copper spray remained on the surface, except for traces. However, De Ong's mixture of copper resinate in pine oil was no more effective than bordeaux against blue mold. The writers recognize that blue mold in many respects behaves very differently from other downy mildews of vegetable crops. Probably the usual type of copper spray mixture, such as bordeaux, and applications designed to coat all exposed plant surfaces with a protective layer are generally the most practical. Certainly, however, this type of spray mixture was not effective against blue mold. The writers' findings suggest that concentration of spray investigations on materials known to be highly toxic to fungus spores may lead to valuable chemotherapeutic agents being overlooked. This is further supported by recent developments in the field of medical pathology. Thus, Long and Bliss (*10*) stated, with regard to such materials as sulfanilamide and sulfapyridine, that no simple explanation has been evolved as to their mode of action. Again, Findlay (*5*) concluded that there is no reason to suppose that chaulmoogra oil acts directly on the causal organism of leprosy, and there is evidence that the condition of the patient has a bearing on the success of the treatment. This latter observation fits in with observations on oil protection against blue mold. Thus, tobacco plants growing in the open, exposed to bright sun, are almost perfectly protected against blue mold by the copper-oil spray; on the other hand, plants that are shaded or grown in the greenhouse in midwinter are much less effectively protected by the spray treatment.

Search for spray mixtures superior to the copper oxide-cottonseed oil combination has recently been centered on the salicylates. A number of these compounds gave marked blue mold control, but the best results have been obtained with benzyl salicylate used with 1 percent oil, and bismuth salicylate without oil. The salicylate sprays have shown superior disease control, and a notable feature has been the prolonged nature of the protection. Bismuth salicylate, which gave excellent blue mold control when used alone, in contrast

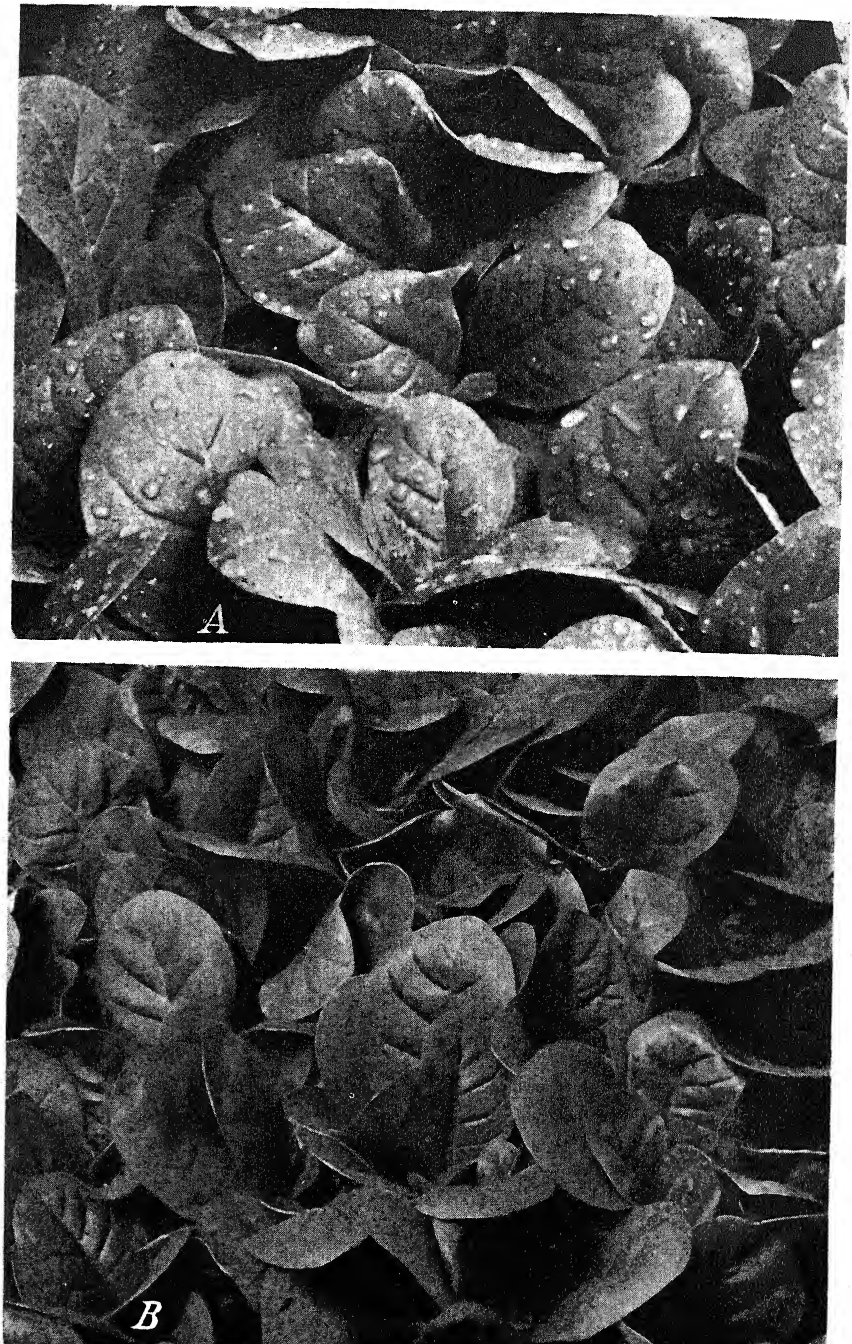

FIGURE 4.—Plants in (*A*) unsprayed plot and (*B*) plot sprayed with copper-oil mixture, about 2 hours after a rain. The oil-sprayed plants dried more quickly than the unsprayed plants, and the sprayed leaves were a much darker green.

to cuprous oxide, which is ineffective without the oil, was only ¼ to 1/16 as toxic to the spores of *Peronospora tabacina* as cuprous oxide. Thus it was again indicated that fungicidal value could not be measured in terms of ability to inhibit spore germination.

SUMMARY

Emulsified oils used as sprays differed greatly in their effectiveness against the blue mold disease of tobacco.

Soybean, linseed, cottonseed, tung, oiticica, and peanut oils were strongly fungicidal. Olive, castor, palm, coconut, chaulmoogra, beef tallow, pine, and paraffin oils were nonfungicidal.

Fungicidal properties appeared to be associated with the presence of linoleic, linolenic, eleostearic, and licanic glycerides.

The tobacco leaves sprayed with a fungicidal oil showed numerous oil globules in the intercellular spaces, and such leaves were resistant to fungal invasion.

A mixture of cottonseed or other fungicidal oil with a copper compound gave more effective blue mold control than the oil alone. Copper oxide alone was almost completely ineffective.

The only emulsifiers that proved satisfactory with copper and oil mixtures were a few of the complex sulfonated alcohol type.

In a search for fungicides that could be used in place of either or both copper and oil, 122 organic compounds were tested.

The salicylates showed strong fungicidal value against blue mold; the most effective were benzyl salicylate with oil, and bismuth salicylate without oil.

Spore-germination tests showed (1) that cuprous oxide inhibited the germination of spores at ⅛ spray strength, (2) that bismuth salicylate was only ¼ to 1/16 as toxic as cuprous oxide, and (3) that cottonseed oil was nontoxic at full spray strength. The spore-germination tests consequently gave no indication of the disease-control value of the several sprays as shown by previous experiments.

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FUNGICIDAL AND PHYTOCIDAL PROPERTIES OF SOME METAL DIALKYL DITHIOCARBAMATES [1]

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INTRODUCTION

The insecticidal value of some metal dialkyl dithiocarbamates has been determined by Guy (*5*),[2] but little information on their fungicidal and phytocidal properties has been published. As early as 1929 Hand (*6*) patented the manufacture and the insecticidal uses of compounds formed by the union of carbon disulfide and organic bases or amines to form the grouping $\underset{\underset{S}{\|}}{-C-}$, and in 1934 Tisdale and Williams (*10*) patented the use of the derivatives of dithiocarbamic acids as disinfectants. In 1927 Cadwell (*1*) patented the use of normal butyl thiocarbonic acid disulfide as a deodorant and in 1929 (*2*) the process of making the substituted dithiocarbamates. In none of the above references were there reports of experiments on the effectiveness of the dithiocarbamic acid derivatives against fungi growing on plants.

Moore, Montgomery, and Shaw (*9*), Montgomery and Moore (*8*), and Marsh (*7*) reported experiments in which derivatives of dithiocarbamic acid were used as fungicides on plants. All these investigators used the thiuram disulfides, and Marsh used zinc diethyl dithiocarbamate in addition. The thiuram disulfides were better fungicides than zinc diethyl dithiocarbamate and were not injurious to apple. In this paper are reported the results of experiments to determine the fungicidal properties of various metal salts of some dialkyl dithiocarbamates and their effects when sprayed on plants.

MATERIALS AND METHODS

Some of the dithiocarbamates, which are commonly used in the vulcanization of rubber, were supplied by the R. T. Vanderbilt Co., of New York, N. Y., and E. I. du Pont de Nemours & Co., Inc., of Wilmington, Del.; others were made in the laboratories of the United States Horticultural Station, Beltsville, Md. For the field tests described in this paper the iron and lead dimethyl dithiocarbamates were furnished by E. I. du Pont de Nemours & Co., and the R. T. Vanderbilt Co., respectively.

The substituted dithiocarbamates are made by treatment of the substituted primary and secondary aliphatic and aromatic amines with carbon disulfide in alkaline alcoholic solutions. As an example,

[1] Received for publication June 9, 1942.

[2] Italic numbers in parentheses, refer to Literature Cited, p. 291.

dimethyl amine in alcoholic solution, when treated with carbon disulfide and sodium carbonate, yields sodium dimethyl dithiocarbamate. One mole of an amine $\begin{array}{c}X\\Y\end{array}\!\!>N{-}H$ combines with one mole of carbon disulfide to form the dithiocarbamic acid $\begin{array}{c}X\\Y\end{array}\!\!>N{-}\underset{\underset{S}{\|}}{C}{-}S{-}H$. X and Y may represent hydrogen or an alkyl radical. The hydrogen attached to sulfur dissociates and may be replaced by sodium to form a soluble dithiocarbamate or by the heavy metals to form insoluble dithiocarbamates, $\begin{array}{c}X\\Y\end{array}\!\!>N{-}\underset{\underset{S}{\|}}{C}{-}S{-}Z$. Z may be a metal or any salt-forming organic or inorganic radical. A considerable range of physical properties is made available by changing the substituents at X, Y, and Z. According to Hand (*6*) and Tisdale and Williams (*10*), the essential pesticide grouping of these compounds is $>N{-}\underset{\underset{S}{\|}}{C}{-}S{-}$.

PHYSICAL PROPERTIES OF TESTED METAL DIALKYL DITHIOCARBAMATES

Some of the physical properties of the various metal dialkyl dithiocarbamates are shown in table 1. Those obtained from the rubber-chemical industry did not differ materially from their counterparts prepared by the writers. Aqueous solutions of the soluble sodium salts, as received, exhibited an alkaline reaction, while derivatives of the heavy metals were acid or neutral. For the most part, the heavy metal salts were very slightly soluble in distilled water. The dimethyl salts were usually more soluble than those of the diethyl and dibutyl series, and the iron and zinc salts of the dimethyl series were considerably more soluble than the others of that group. Most of the metal dialkyl dithiocarbamates were easily suspended in either distilled or tap water. None of the compounds made up in the writers' laboratories possessed any particular odor. The commercial sodium compounds used by the writers as reagents for the formation of metal derivatives [3] possessed a strong disagreeable odor. Since the dimethyl and diethyl metal dithiocarbamates obtained from the chemical companies also possessed this same odor, it is suggested that they had not been freed of the sodium reagent used in their preparation. On standing in contact with air, the iron dimethyl dithiocarbamate prepared by the writers generated an odor resembling that of carbon disulfide. The butyl derivatives of lead and silver were difficult to make, and their physical properties indicated the presence of impurities. Because of this the data obtained with the silver and lead salts, although included in the tables, are not considered fully reliable.

[3] For suggesting the preparation of the heavy metal salts by mixing solutions of sodium salts with solutions of inorganic salts of heavy metals, the writers are indebted to Paul I. Murrill, formerly chief chemist of the R. T. Vanderbilt Co.

TABLE 1.—*Chemical and physical properties of the metal dialkyl dithiocarbamates tested*

Substituted dithiocarbamate	Source [1]	Physical nature	Melting point (uncorrected)	Appearance	Wettability in water	Solubility in distilled water of room temperature	pH (glass vs. saturated calomel electrode)
			° C.			*P. p. m.*	
Sodium dimethyl	Vanderbilt; Du Pont	Liquid (solid in solution).		Colorless	Complete	>1,000	10.5
Sodium diethyl	Vanderbilt	Solid	85 to 95	White	do	>1,000	10.0
Sodium dibutyl	Vanderbilt; Du Pont	Liquid (solid in solution).		Reddish orange	do	>1,000	9.9
Iron dimethyl	Beltsville; Du Pont	Solid	Dec.[2]	Very dark brown	do	120	5.0
Iron diethyl	Beltsville	do	251	Dark chocolate brown	Difficult	10	5.9
Iron dibutyl	do	do	245	Black; a gray precipitate appeared during preparation, but disappeared later.	Complete	12	7.4
Zinc dimethyl	Beltsville; Vanderbilt	do	246	White	do	65	6.5
Zinc diethyl	Beltsville	do	175	do	Difficult	13	5.6
Zinc dibutyl	Beltsville; Vanderbilt	do	104.5	do	do	52	7.0
Lead dimethyl	do	do	Dec. 305	do	Complete	44	6.4
Lead diethyl	Beltsville	do	204	do	do	22	5.9
Lead dibutyl	do	do	77	Orange yellow turning gray brown on standing; dry pure substance white.	do	18	6.0
Copper dimethyl	Beltsville; Vanderbilt	do	Dec. 305	Brown	do	12	4.3
Copper diethyl	Beltsville	do	201	Chocolate brown	do	12	5.6
Copper dibutyl	do	do	77 [3]	A black hard crystalline substance; suspension that would not settle was discarded.	do	18	4.3
Silver dimethyl	do	do	Dec. 289	Grayish white	do	8	5.3
Silver diethyl	do	do	175	do	do	11	5.5
Silver dibutyl	do	do	107	White; a red and an orange contaminant appearing during preparation removed by recrystallization.	do	18	5.5
Mercury dimethyl	do	do	214	Faintly yellowish white	do	20	5.5
Mercury diethyl	do	do	112	Very light yellow	do	12	5.7
Mercury dibutyl	do	do	101	Dirty white	do	11	5.3
Selenium diethyl	Vanderbilt	do	67	Yellowish white turning drab to brown on handling.	Difficult		6.8

[1] Vanderbilt=R. T. Vanderbilt Co.; Du Pont=E. I. du Pont de Nemours & Co, Inc.; and Beltsville=fungicide laboratory, U. S. Horticultural Station, Beltsville, Md.
[2] Decomposes before melting.
[3] Not sharp; evidence of contamination.

It cannot be taken for granted that carefully weighed equivalents of a soluble dialkyl dithiocarbamate and of a soluble salt of a heavy metal will unite only by replacement of the hydrogen attached to sulfur. In the first place, as shown in table 1, the soluble salts of these compounds display an alkaline reaction when dissolved in water. Furthermore, if one of these solutions is neutralized or acidified the free acid appears as a precipitate. Most of the solutions of salts of heavy metals that would be used to prepare the heavy metal dialkyl dithiocarbamates are neutral if not acid, and basic salts are precipitated if these solutions are neutralized or made alkaline. Accordingly the solutions intended for the preparation of a desired heavy metal dialkyl dithiocarbamate must be made up as nearly neutral as possible and must be mixed with all customary precautions to avoid inclusions, entrainment, and local exhaustion of reactants. Even with the best of care some contamination with basic salts of the heavy metals and dialkyl dithiocarbamic acid is to be expected. In the laboratory the solution of the sodium salt was run into the solution of the heavy metal salt slowly and with thorough stirring. Extraction with an organic solvent would then remove the basic salts.

In the second place the dialkyl dithiocarbamates oxidize very readily. The most usual course is the elimination of the dissociable hydrogen from each of two molecules, which then combine to form a tetraalkyl thiuram disulfide. This product is insoluble and appears not to combine with metals. This propensity makes difficult the preparation of heavy metal salts in their highest state of oxidation, for example, ferric dialkyl dithiocarbamates.

Examinations of the iron salts used in this study have raised doubts regarding their composition. On storage also these materials gave evidence of changes. Conflicting reports from workers who have tested these substances in the field have given the impression that their supplies were not alike. It is suggested that even though a solution of ferrous sulfate is used in the preparation, oxidation of the organic residue takes place to some extent. Attempts to separate by extraction a substance with the theoretical iron content will not be reported here. Supplies of iron dialkyl dithiocarbamates used by others for insecticidal and fungicidal tests are probably similar in composition to those studied by the writers.

EXPERIMENTAL PROCEDURES AND CONDITIONS

The phytocidal properties of the various compounds were determined by spraying Red Kidney bean (*Phaseolus vulgaris* L.) and various varieties of peach (*Amygdalus persica* L.) and of apple (*Malus pumila* Mill.), all in vigorous condition. (See table 2.) The dialkyl dithiocarbamates, when applied to apple, were combined with lime and lead arsenate at the rate of 2 pounds of the metal dialkyl dithiocarbamate, 8 pounds of hydrated lime, and 2 pounds of acid lead arsenate to 100 gallons of spray fluid. The lead arsenate was added for the control of insect pests and the lime as a "safener" against arsenical injury, since it was not known whether the dialkyl dithiocarbamates possessed insecticidal properties. On bean and peach, the dialkyl dithiocarbamates were used with lime but without lead arsenate. The sprays were applied to bean and small limbs of peach and apple with a hand sprayer several times during the season. Only

in the amount of pressure used did these small-scale experiments differ from the more extensive tests reported on page 287.

The fungicidal properties were determined according to the methods outlined by Goldsworthy and Green (*3*, *4*). *Sclerotinia fructicola* (Wint.) Rehm (the peach brown rot pathogen) and *Glomerella cingulata* (Ston.) Spauld. and Von Schrenk (the apple bitter rot pathogen) grown under standardized environmental conditions were used as the test organisms. The tests included subjecting conidia to saturated distilled-water solutions of the materials during a 24-hour perfusion period, and to residues from the chemicals, with and without adjuvants, sprayed on glass cover slips and allowed to weather.

The perfusion tests (*4*) were conducted with solutions obtained by saturating 2 liters of distilled water with an excess of the chemical. Not less than 7 days was allowed for the solid and solvent to reach equilibrium. The equilibrium solution thus obtained was then passed through a special perfusion apparatus so that it came in contact with conidia in sterile water-agar cubes. During the 24-hour perfusion period, tests of the fungicidal activity of these solutions were usually made at the end of 2, 4, 6, 22, and 24 hours. Each test was made by removing one of the conidia-containing agar cubes from the perfusion cells, examining one half of it under the microscope for conidial germination, and placing the other half on sterile oxidized potato-juice 4-percent agar to test growth capability. Observations on germination were recorded at the time the cubes were taken out of the equilibrium solution and after a 24-hour incubation period on the potato agar. (See table 4.)

In 1941 spray-residue tests (see table 2) were conducted in the following manner. The chemicals used were mixed at the rate of 2 pounds of the test material, 8 pounds of hydrated lime, and 2 pounds of lead arsenate to 100 gallons of spray fluid, and the mixture was sprayed on thin glass cover slips. The spraying of the agitated mixture on the glass cover slips was done at a standard pressure and for a given time, and the resulting residues represent similar loads, at least for any given mixture. For each chemical 12 cover slips were prepared as indicated. These were then suspended in unsprayed apple trees by means of special holders so that rain, dew, wind, temperature changes, leaf excretions, insects, fungi, light, carbon dioxide concentration, and other factors could operate and perhaps change their physical and chemical properties from day to day. Some of the glass cover slips were brought into the laboratory every other day to test the fungicidal properties of the residues. This was done by determining their degree of toxicity to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* by a standardized procedure (*3*). Since the testing period usually lasted about 2 weeks (a length of time often elapsing between applications of orchard sprays), seven separate tests of a residue were made during that time. All these tests were made during the regular spraying season, and the results are somewhat indicative of what the materials will do under orchard conditions.

Rainfall and temperature are not recorded in table 2, since space does not permit the inclusion of the continuous records obtained during the tests. The experiments summarized in table 2 were conducted during three separate 2-week periods. During these periods temperatures were mostly moderate, and 1.5, 3.4, and 1.84 inches of

rainfall were recorded for the respective periods. In all of these tests the residues were subjected to precipitation and other weather conditions sufficient to provide a rigid test of their sticking and weathering properties.

The fungicidal and phytocidal properties of combinations obtained by tank mixing of sodium dimethyl dithiocarbamate with ferrous sulfate, zinc sulfate, and lead acetate, respectively, to form ferric, zinc, and lead dimethyl dithiocarbamates were tested. (See table 3.) The combinations were used alone and in some cases with lead arsenate, lime, lead arsenate and lime, bentonite, bentonite and lime, or bentonite and nicotine sulfate, which have been used in combination for control of the codling moth.[4] These tests were made during three 2-week periods in which 1.84, 1.55, and 2.18 inches of rainfall, respectively, were recorded. Temperatures were mostly moderate.

During the 1941 season field tests were conducted on apple, peach, and cherry at the United States Horticultural Station, Beltsville, Md., and on apple at Mountain Grove, Mo., with a commercial sample of ferric dimethyl dithiocarbamate. Lead dimethyl dithiocarbamates, consisting of a sample of the pure material and of one in which the lead compound was diluted 70 percent with china clay, were also tested on apple at the Maryland station.

The season was unfavorable for the production of apple scab, caused by *Venturia inaequalis* (Cke.) Wint., at the Maryland station, but favorable for the disease at the Missouri station. At the Maryland station cherry leaf spot, caused by *Coccomyces hiemalis* Hig., was plentiful; and the season was favorable for peach scab, caused by *Cladosporium carpophilum* Thüm., but not for brown rot, caused by *Sclerotina fructicola*. In Maryland the growing season was marked by an absence of rainfall at the beginning and end and by an abundance of rainfall during the remainder. The temperatures, as a whole, were moderate, sometimes rather cool but seldom hot (fig. 1). The season was favorable for the production of copper and arsenical injury, but not for sulfur injury. The effect of drought was extreme by the end of the growing season. In Missouri the early and middle parts of the growing season were marked by a general abundance of rainfall accompanied by moderate temperatures. Rainfall was 7.67 inches in April, 4.07 in May, 2.86 in June, 6.17 in July, 1.01 in August, and more than 10 in September.

EXPERIMENTAL RESULTS

PERFUSION AND SPRAY-RESIDUE TESTS

The phytocidal data presented in table 2 show that the lead dialkyl dithiocarbamates tested were safely applied to all the plants used. Of the iron compounds tested, the dimethyl compound caused injury to peach. This injury developed after a period of weathering, indicating that this material was unstable. None of the zinc compounds tested was injurious to apple, but the dimethyl and diethyl compounds caused delayed injury to bean foliage; on peach the diethyl compound of zinc caused injury of the same type as the ethyl compound of iron but of a lesser degree. All of the copper, selenium, and mercury compounds used proved to be injurious to the test plants,

[4] STEINER, L. F., SAZAMA, R. F., FAHEY, J. E., and RUSK, H. W. TANK-MIX NICOTINE-BENTONITE FOR CONTROL OF THE CODLING MOTH. U. S. Bur. Ent. and Plant Quar. Cir. E-428. [Processed.]

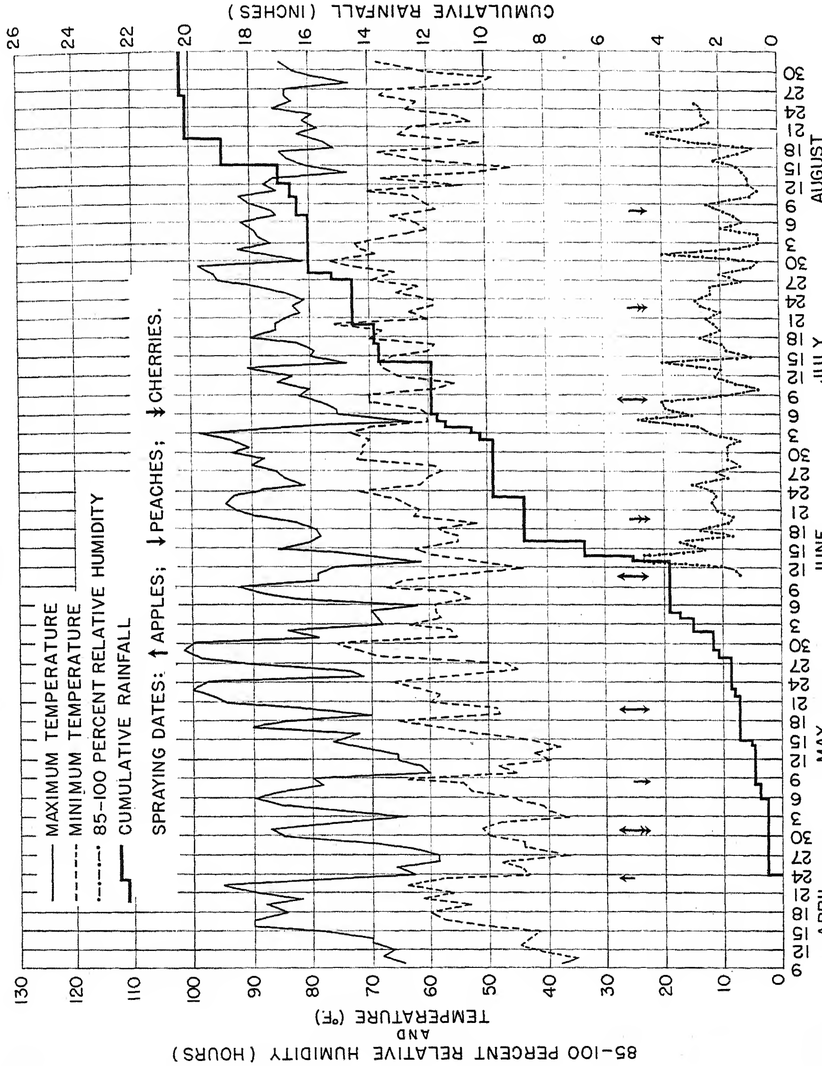

FIGURE 1.—A graphic summary of relative humidity, rainfall, temperature, and spraying dates at the United States Horticultural Station, Beltsville, Md.

and the silver dibutyl compound was injurious to bean and peach. The mercury compounds proved to be the most injurious of those tested. The injury caused by the iron and zinc compounds may properly be described as a leaf-spotting or shot-hole effect. Very little marginal leaf injury and no bark or fruit symptoms were noted. The injury caused by the silver, selenium, copper, and mercury compounds, on the other hand, was of a more pronounced character. The leaves of peach and bean and the bark of young peach twigs were severely injured; not only leaves spotted, but marginal injury, yellowing, and leaf fall were common. On apple, spot necrosis, marginal necrosis, and yellowing of leaves were common, but bark injury and leaf fall did not occur. On peach fruits necrosis was observed where moisture accumulated, and on apple fruits a superficial russeting developed. None of the metal dialkyl dithiocarbamates prevented the typical arsenical injury from developing on the leaves and bark of peach when lead arsenate was present in the mixture. The zinc compounds appeared to cause the least of this type of injury.

TABLE 2.—*Small-scale tests of phytocidal and fungicidal properties of some metal dialkyl dithiocarbamates*

Substituted dithiocarbamates[1]		Injury caused by spray residue to—			Duration[3] of toxicity of spray residues[2] to—	
Metal	Alkyl group	Bean	Apple[2]	Peach	*Sclerotinia fructicola*	*Glomerella cingulata*
					Days	*Days*
Sodium	Dimethyl	Severe				
	Dimethyl (with lime).		None	None	13	13
	Diethyl (with lime).		None	None	0	0
	Dibutyl	Moderate		Moderate		
	Dibutyl (with lime).		None	None	0	0
Ferric	Dimethyl	None	do	Slight	13	13
	Diethyl	do	do	None	0	0
	Dibutyl	do	do	do	0	0
Zinc	Dimethyl	Moderate	do	do	13	13
	Diethyl	do	do	Moderate	4	4
	Dibutyl	None	do	None	0	0
Lead	Dimethyl	do	do	do	13	13
	Diethyl	do	do	do	6	6
	Dibutyl	do	do	do	0	0
Copper	Dimethyl	Moderate	Moderate	Moderate	13	13
	Diethyl	do	do	do	6	6
	Dibutyl	do	do	do	0	0
Silver	Dimethyl	None	None	None	13	13
	Diethyl	do	do	do	13	13
	Dibutyl	Moderate	do	Moderate	0	0
Mercury	Dimethyl	do	Moderate	do	13	13
	Diethyl	do	do	do	13	13
	Dibutyl	do	do	do	13	13
Selenium	Diethyl	do	do	do	13	13

[1] In all these experiments heavy metal dialkyl dithiocarbamates were used at the rate of 2 pounds with 8 pounds of lime per 100 gallons of spray fluid. The sodium salt with or without lime was used at the same strength.

[2] Lead arsenate at the rate of 2 pounds to 100 gallons of spray fluid was added for insect control on apples and in tests of toxicity of spray residue to conidia.

[3] Testing period lasted 2 weeks.

In the experiments presented in table 3, where the metal dimethyl dithiocarbamates were made up in the spray tank by mixing stoichiometrical equivalents of the reacting soluble salts, no injury resulted from the use of the lead combinations, but the zinc and iron salts caused injuries similar to those listed in table 2. When the tank-mixed

iron salt was combined with bentonite only, no injury developed even on peach. When the sodium dimethyl dithiocarbamate was used in excess injury developed on peach, but if the iron sulfate was in excess no injury developed (table 3). Combinations of tank-mixed iron dimethyl dithiocarbamate, nicotine sulfate, and bentonite, on the other hand, caused injuries on peach similar to the mixture containing lime.

These data indicate that the iron, zinc, and lead salts may be safely applied to apple and that the lead salts may be safely applied to peach. None of the others appear promising.

TABLE 3.—*Small-scale orchard tests of fungicidal and phytocidal properties of iron, zinc, and lead dimethyl dithiocarbamate spray combinations with various adjuvants*

Combination (No. and description)	Rainfall during test	Injury caused by spray residue to—		Duration of toxicity of residues to—	
		Apple	Peach	*Sclerotinia fructicola*	*Glomerella cingulata*
	Inches			*Days*	*Days*
1. Tank-mix iron salt [1]	1.84	None	Slight	12	12
2. No. 1 + lead arsenate (2 lb.)	1.84	do	do	12	12
3. No. 1+lead arsenate (2 lb.)+hydrated lime (8 lb.)	1.84	do	do	12	12
4. No. 1+hydrated lime (8 lb.)+bentonite (2 lb.)	1.84	do	do	4	4
5. No. 1+bentonite (4 lb.)+nicotine sulfate (1 pt.) added in order given (A, B, C, and D).[2]	2.18	do	do	13	13
6. Same as No. 5 but materials added in the order B, A, C, and D.[2]	2.18	do	do	13	13
7. Same as No. 5 but materials added in the order C, A, B, and D.[2]	2.18	do	do	13	13
8. Same as No. 5 but materials added in the order C, B, A, and D.[2]	2.18	do	Considerable.	13	13
9. No. 1 (half strength)+bentonite (5 lb.)	2.18	do	None	13	13
10. No. 9+excess sodium dimethyl dithiocarbamate (0.5 lb.).	2.18	do	Slight	13	13
11. No. 9+excess ferrous sulfate (0.5 lb.)	2.18	do	None	13	13
12. Tank-mix zinc salt [3]	1.55	do	Slight	13	13
13. Tank-mix lead salt [4]	1.55	do	None	13	13
14. No. 13+hydrated lime (4 lb.)	1.55	do	do	13	13
15. No. 13+hydrated lime (4 lb.)+lead arsenate (2 lb.)	1.55	do	Slight	13	13
16. Clay lead salt mixture [5]+hydrated lime (4 lb.)	1.55	do	None	11	13
17. No. 16+lead arsenate (2 lb.)	1.55	do	Slight	11	11

[1] Stoichiometrical quantities of ferrous sulfate and sodium dimethyl dithiocarbamate were mixed to give ferric dimethyl dithiocarbamate at the rate of 2 pounds per 100 gallons of spray fluid.

[2] In the tests (Nos. 5, 6, 7, 8) of the effect of changing the order of adding ingredients 2 pounds of ferrous sulfate=A; 2 pounds of sodium dimethyl dithiocarbamate=B; 4 pounds of bentonite=C; 1 pint of 40 percent nicotine sulfate solution=D.

[3] As in No. 1, the required quantities of zinc sulfate and sodium dimethyl dithiocarbamate were mixed in water to give zinc dimethyl dithiocarbamate at the rate of 2 pounds per 100 gallons of spray fluid.

[4] As in No. 1, the required quantities of lead acetate and sodium dimethyl dithiocarbamate were mixed in water to give lead dimethyl dithiocarbamate at the rate of 2 pounds per 100 gallons of spray fluid.

[5] A commercial preparation containing 30 percent of lead dimethyl dithiocarbamate in a special clay was used at the rate of 4 pounds with 4 pounds of hydrated lime per 100 gallons of spray fluid.

The tests of the fungicidal properties of the residues indicate in general that, of the metal dialkyl dithiocarbamates studied, those of the dimethyl series appear to offer the most promise while those of the dibutyl series offer the least. Of the diethyl series only the selenium, silver, and mercury salts were fully effective, but the zinc, lead, and copper compounds were partly so (table 2). The addition of lime, as a carrier of the dialkyl dithiocarbamates, or of a combination of lime and of lead arsenate, and lime, as a "safener" for the lead arsenate appeared not to affect the fungicidal properties of the dialkyl dithio-

carbamates. The addition of bentonite or of bentonite flocculated by nicotine sulfate likewise failed to reduce the fungicidal efficiency of these compounds. The addition of bentonite flocculated by lime appeared to reduce their fungicidal properties (table 3).

In tests, not recorded in the tables, longer periods of weathering appeared to reduce the fungicidal value of the iron, zinc, and lead dimethyl dithiocarbamates even though considerable quantities of the residue remained on the cover slips. This appeared most pronounced with the iron compound, less with the zinc, and least with the lead.

In the perfusion tests (table 4) where the materials were used without the addition of other chemicals, in general the dimethyl salts were the most effective fungicides, followed in order by those of the diethyl and dibutyl series. This order was maintained with the sodium series.

TABLE 4.—*Tests of toxicity of saturated solutions of some metal dialkyl dithiocarbamates to conidia*

Substituted dithiocarbamates		*Sclerotinia fructicola*		*Glomerella cingulata*	
Metal	Alkyl group	Conidia killed	Perfusion period	Conidia killed	Perfusion period
		Percent	*Hours*	*Percent*	*Hours*
Sodium	Dimethyl	100	2	100	2
	Diethyl	100	22	100	4
	Dibutyl	99+	24	99+	24
Ferric	Dimethyl	100	2	100	2
	Diethyl	48	24	32	24
	Dibutyl	0	24	0	24
Zinc	Dimethyl	100	4	100	4
	Diethyl	0	24	0	24
	Dibutyl	0	24	0	24
Lead	Dimethyl	100	4	100	2
	Diethyl	100	24	100	4
	Dibutyl	20	24	50	24
Copper	Dimethyl	100	6	----	----
	----	100	2	100	2
	Diethyl	100	22	100	22
	Dibutyl	0	24	0	24
Silver	Dimethyl	100	22	0	24
	Diethyl	100	2	100	2
	Dibutyl	100	22	100	4
Mercury	Dimethyl	100	2	100	2
	Diethyl	100	2	100	2
	Dibutyl	100	2	100	2
Selenium	Diethyl	100	2	100	2

The present study does not indicate the grouping in the metal dialkyl dithiocarbamate molecule that is responsible for either the phytocidal or the fungicidal behavior of the various compounds. Where copper, selenium, mercury, or silver is part of the molecule, it seems certain that these could exercise considerable influence on both properties. Where iron, lead, or zinc is concerned the answer becomes hard to find. As stated previously, Hand (*6*) and Tisdale and Williams (*10*) were of the opinion that all these compounds are of value as fungicides or insecticides because of the presence of the characteristic group $>N-\underset{\underset{S}{\|}}{C}-S-$. The phytocidal data appear to indicate that this group, per se, is not responsible for plant injury; the fungicidal data do not clearly indicate that this group, per se, is responsible for the

fungicidal properties. All the compounds possess this grouping, yet many possess no fungicidal properties when used alone or with adjuvants. There is some indication that the fungicidal property may be correlated with solubility, but a few contradictory results obtained with some of the compounds more or less invalidate this theory. This is particularly true of the more soluble dialkyl dithiocarbamates. Among these the fungicidal property was greatest with the methyl compound and least with the butyl, yet all were completely soluble at the concentration used. The data appear to indicate that in addition to the effect, if any, of the characteristic disulfide group, or a byproduct of this group (CS_2), the character of the dialkyl groups may modify the fungicidal property. This was greatest with the dimethyl and least with the dibutyl series.

FIELD TESTS

Experiments for the Control of Apple Scab

Table 5 shows the results of spraying with ferric dimethyl dithiocarbamate at the Missouri station for the control of apple scab and the prevention of fruit russet. Apple scab was well controlled without any noticeable effect on fruit vigor, finish, or color. At the Maryland station ferric and lead dimethyl dithiocarbamates were sprayed on Delicious, Stayman Winesap, Grimes Golden, Golden Delicious, and York Imperial apple varieties, but because of the dry weather in the early part of the season no fruit scab and only a moderate amount of leaf scab developed on the check trees.

At the Maryland station during the early dry warm season both the iron and the lead compound caused a small amount of spot necrosis between the veins of the leaves of all varieties, but this injury was so slight that at the end of the season none could be detected. The spray residues were uniform, weathered well, and did not inferfere with the normal coloring of the fruit of any apple varieties tested. The residues of the lead compounds were the most tenacious. The materials proved rather easy to handle at both stations and caused no harm to the machinery or to the operators. At neither station was russet a factor, and a fine finish developed on all varieties regardless of treatment.

TABLE 5.—*Comparison of control of apple scab and russet by ferric dimethyl dithiocarbamate and by standard fungicides, Mountain Grove, Mo.*

Treatment	Variety	Drops			Harvested fruit			Total crop		
		Total	Scabbed	Russeted	Total	Scabbed	Russeted	Sound	Scabbed	Russeted
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*
No fungicide [1]	Rome Beauty	9,596	9,184	<10	2,071	1,917	<10	4.9	95.1	<1
Ferric dimethyl dithiocarbamate mixture+lead arsenate.[2]	do	5,501	216	<10	1,264	31	<10	96.9	3.2	<1
Liquid lime-sulfur; bordeaux mixture; lead arsenate.[3]	do	1,477	14	<10	672	5	<10	99.2	.8	<1
No fungicide [1]	Golden Delicious	1,051	1,050	12	390	389	9	<.1	99.9	1.4
Ferric dimethyl dithiocarbamate mixture+lead arsenate.[2]	do	163	4	2	171	5	8	97.4	2.6	2.9
Liquid lime-sulfur; wettable sulfur, hydrated lime, lead arsenate.[4]	do	188	1	4	278	0	5	99.9	.1	1.9
Lead arsenate, hydrated lime [5]	do	509	276	42	643	397	54	41.6	58.4	8.3

[1] 4 pounds of hydrated lime and 2 pounds of lead arsenate per 100 gallons of spray fluid (3 cover applications).
[2] 3 pounds of ferric dimethyl dithiocarbamate, 4 pounds of hydrated lime, and 2 pounds of lead arsenate per 100 gallons of spray fluid (3 blossom and 4 cover applications).
[3] 2.5 gallons of liquid lime-sulfur to 100 gallons of spray fluid (3 blossom and 1 cover application); 4-6-100 bordeaux mixture (3 cover applications); 2-100 lead arsenate (1 calyx and 4 cover applications).
[4] 2.5 gallons of liquid lime-sulfur per 100 gallons of spray fluid (3 blossom applications); 8 pounds of wettable sulfur, 4 pounds of hydrated lime, and 2 pounds of lead arsenate to 100 gallons of spray fluid (1 calyx and 4 cover applications).
[5] 4 pounds of hydrated lime and 2 pounds of lead arsenate to 100 gallons of spray fluid (1 calyx and cover applications).

EXPERIMENT FOR THE CONTROL OF PEACH SCAB AND BROWN ROT

Table 6 shows the results of applications of ferric dimethyl dithiocarbamate to peach varieties at the Maryland station. The season was favorable for peach scab, since frequent rains and moderate temperatures occurred during most of the growing season. By harvest-time the fruits of the check trees of the Carman and Elberta varieties were often cracked from the disease. The late season was somewhat dry, and brown rot was not the menace that it usually is at the Maryland station. Only a trace of scab and very little brown rot developed on the sprayed Alexander and Carman varieties. Only a trace of brown rot and 9.2 percent of scab, all of a light character and insufficient to cause a loss of grade, developed on the Elberta variety. The fruit developed a fine finish in all cases. The material, however, had a physiological effect upon the ripening processes that delayed maturity of all varieties from 7 to 10 days and likewise interfered somewhat with the development of color. The material caused no noticeable blemishes on the fruit of the various varieties, but on all varieties certain leaf injuries occurred throughout the season. After the first two applications, in which zinc sulfate was combined with the mixture to prevent injury by the lead arsenate, a few necrotic spots developed between the veins of the younger leaves. These were noticeable several days after the applications but soon became hard to find as new foliage developed. When the later cover applications were made without zinc sulfate and lead arsenate, this type of injury developed in increasing amounts. It appeared that the material had become changed as it aged in the warehouse. In the last application, the injury was very severe, but it resulted in little defoliation.

TABLE 6.—*Control of peach scab and brown rot by ferric dimethyl dithiocarbamate, Beltsville, Md.*

Treatment	Variety	Fruit counted	Scab		Fruit affected by brown rot	Fruit affected by worms
			Fruit affected	Severity on affected fruit		
		Number	*Percent*		*Percent*	*Percent*
Ferric dimethyl dithiocarbamate mixture.[1]	Alexander	467	<1.0	Light	<1.0	16.7
No treatment	do	610	89.9	Heavy	3.9	43.0
Ferric dimethyl dithiocarbamate mixture.[1]	Carman	319	<1.0	Light	5.0	16.8
No treatment	do	666	100	Heavy	21.5	36.9
Ferric dimethyl dithiocarbamate mixture.[1]	Elberta	1,432	9.2	Light	<1.0	23.1
No treatment	do	1,231	100	Heavy	40.5	68.3

[1] 2 pounds of ferric dimethyl dithiocarbamate and 8 pounds of hydrated lime per 100 gallons of spray fluid (5 applications); 2 pounds of lead arsenate and 2 pounds of zinc sulfate added to shuck and first cover, but not to second, third, and fourth cover, applications.

EXPERIMENTS FOR THE CONTROL OF CHERRY LEAF SPOT

Table 7 shows the results of applications of ferric dimethyl dithiocarbamate to the Montmorency variety of cherry at Beltsville, Md. The early part of the season was not particularly favorable for the development of cherry leaf spot, but considerable leaf spot developed as the season advanced. At harvesttime development from tree to

tree was not uniform, but as the season advanced the disease spread rapidly through the planting, subjecting the materials to a thorough test. Many of the trees were bare at the beginning of October, some weeks before the advent of frost. No injury resulted from the treatments, but leaf spot was not controlled.

TABLE 7.—*Comparison of control of leaf spot of Montmorency cherries by ferric dimethyl dithiocarbamate and by copper phosphate, Beltsville, Md.*

Treatment [1]	Trees	Average size of fruit at harvest [2]	Leaf infection (average of all trees in plot) on—		Leaf fall (average of all trees in plot) on—	
			Aug. 30	Sept. 26	Aug. 30	Sept. 26
	Number	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Ferric dimethyl dithiocarbamate mixture [3]	8	4.31	78.0	100.0	10.0	74.5
Copper phosphate mixture [4]	9	3.79	.45	13.3	0	2.0
No treatment	3	4.06	100.0	100.0	80.0	96.0

[1] 1 petal-fall and 2 cover applications.
[2] About 2 pounds of fruit was collected from each tree by harvesting the fruit around the tree at breast height.
[3] 2 pounds of ferric dimethyl dithiocarbamate, 8 pounds of lime, and 2 pounds of lead arsenate per 100 gallons of spray fluid at petal fall; same without lead arsenate in 2 cover applications.
[4] 2 pounds of copper phosphate, 8 pounds of lime, and 2 pounds of lead arsenate per 100 gallons of spray fluid at petal fall; same without lead arsenate in 2 cover applications.

SUMMARY

Results of experiments to determine the fungicidal and phytocidal properties of some metal dialkyl dithiocarbamates, including the sodium, ferric, lead, zinc, copper, silver, and mercury dimethyl, diethyl, and dibutyl dithiocarbamates and selenium diethyl dithiocarbamate, are reported.

Most of the heavy metal dialkyl dithiocarbamates were produced in the laboratories of the United States Horticultural Station, Beltsville, Md., by reacting solutions of commercial sodium dialkyl salts with solutions of suitable heavy metal salts.

The sodium salts have a basic reaction while the salts of the heavy metals are acid or neutral. The soluble sodium salts were phytocidal. The selenium, copper, and mercury salts were all phytocidal to all the various plants used, while those of iron, zinc, and silver varied in this respect. The lead salts caused no injury to any of the plants, while the zinc salts caused more or less injury to peach and bean and the dimethyl salt of iron caused slight injury to peach. Apparently iron and zinc salts become changed by weathering.

The dimethyl derivatives appeared in general to possess the greatest fungicidal value. The dibutyl derivatives in general possessed the least. The iron, lead, and zinc dimethyl derivatives appeared to offer the greatest promise since these caused the least plant injury. The lead salts appear the most promising from all standpoints.

Preliminary tests indicated that the iron, zinc, and lead derivatives can be made up in the spray tank by mixing stoichiometrical equivalents of the reacting salts. Such an operation results in a product that remains in suspension and has superior sticking qualities. The zinc and iron mixtures of this type caused slight injuries to peach, while none caused injury to apple.

All of the metal dialkyl dithiocarbamates were compatible with hydrated lime and lead arsenate. Special tests with the iron dimethyl

dithiocarbamates indicated that this metal derivative retained its fungidicidal efficiency where combined with bentonite or nicotine sulfate and bentonite but not with bentonite flocculated with lime.

Field tests were conducted in Maryland and Missouri on a small scale with ferric dimethyl dithiocarbamate for the control of apple scab. The product used was a commercial sample. In spite of the fact that there was little scab at the Maryland station the results were satisfactory, and the material showed enough promise to justify continuing the tests. The material controlled scab and brown rot on peach in Maryland without causing fruit injury, but it caused leaf spotting which became progressively greater as the material on the leaves aged. On cherry, no injury occurred, but leaf spot was not controlled.

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RELATION OF GREEN LINT TO LINT INDEX IN UPLAND COTTON [1]

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INTRODUCTION

Cotton breeders and producers of cotton have long been interested in obtaining commercial varieties that possess a combination of high lint percentage and other desirable characters. The exact mode of inheritance of lint abundance is not understood, presumably because it is a compound character and each of the functions affecting it is in turn inherently complex and affected by a number of environmental influences. A more thorough understanding of the mode of inheritance of the capacity of a cotton plant to produce a characteristic quantity of lint on the individual seed should be of value to breeders who are working along these lines of improvement.

GREEN-LINT CHARACTER

The cotton fiber, being an extended epidermal cell, is a unicellular structure. The number of fibers arising from the epidermal layer of a single seed of the commercial upland cottons has been estimated as between 10,000 and 20,000. Commercial cotton fibers are white or light cream. In several noncommercial varieties, brown fibers of various shades are found, and at least one noncommercial variety is characterized by green fibers. The green pigment may be seen soon after the fibers begin to thicken and is apparent by 25 days after flowering. As the fibers develop, the color is intensified until the boll opens, displaying the bright-green pigmentation. On exposure to light the color gradually fades to a brownish green. Kerr has found that the green pigment occurs in the cell wall and that this coloration is not present in the protoplasmic contents of the cell.[3]

Harland (*6*) [4] and Ware (*24*) have reported that green lint is determined primarily by a single pair of genetic factors, to which the symbols *Lg*, *lg* have been assigned by Hutchinson and Silow (*9*). Green is incompletely dominant over white, the F_1 being characterized by a reduced amount of color. The F_2 segregation consists of green, intermediate green, and white segregates in a ratio of 1 : 2 : 1. Progenies of the F_2 green and white phenotypes breed true in the F_3, and progenies of the F_2 intermediate-green phenotypes again segregate into three classes. Segregation in the study here presented conformed satisfactorily to the monofactor hypothesis, since a popu-

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[3] The writer is grateful to Thomas Kerr, of this Division, for permission to refer to this heretofore unpublished information.

[4] Italic numbers in parentheses refer to Literature Cited, p. 305.

lation segregating in the ratio of 113 green to 228 intermediate green to 116 white was obtained in the F_2.

LINT-PERCENTAGE AND LINT-INDEX CHARACTERS

The writer (*16*) has discussed the lint-percentage and lint-index characters and their reaction to environmental influences. The method of determination has been described by Meloy (*15*).

McLendon (*14*), Kottur (*11*), Kulkarni and Khadilker (*12*), Thadani (*22*), Texas Agricultural Experiment Station workers (*20, pp. 47 and 48*), Ware (*23, 25, 26*), and O'Kelly and Hull (*17*) have published results regarding the mode of inheritance of lint-index and lint-percentage characters in cotton. In studies in which both parental lines were upland cottons with fuzzy seed, the results, as far as can be ascertained, are similar to those obtained in the analysis of quantitative characters. In studies in which one parental line was a high-lint-percentage, fuzzy-seed upland and the other parental line was a low-lint-percentage, naked-seed upland, the investigators have found a segregation in which it appeared that lint percentage was controlled by a single factor pair. In these cases the segregation was that of basic seed-fuzz genes. Thadani (*21, 22*), Kearney and Harrison (*10*), Carver (*2*), Griffee and Ligon (*4*), and Ware (*25, 26*) have reported that naked-seed upland cottons differ from the fuzzy-seed uplands by one factor pair to which has been allotted the symbols F^nf^n by Hutchinson and Silow (*9*). Texas workers (*20, p. 48*) and Ware (*25, 26*) have shown that high lint values are associated with the presence of fuzz and sparse lint with naked seed.

RELATION OF GREEN LINT AND LINT INDEX

Hull (*7*) has called attention to the association between lint color and lint percentage of cotton in the F_2 and backcross populations of a cross between a green-lint strain having a low lint percentage and several white-lint strains having higher lint percentages, and has suggested that the factors that affect lint color are modifiers of lint percentage. Ginning data obtained by the writer and his associates in conducting lint-color inheritance studies provide a verification of this finding.

The purpose of this study was (1) to further demonstrate the relation between the green-lint factor and the lint-index values and (2) to attempt to determine the type of association that exists.

Lindstrom (*13*) has suggested that one of the most direct means of testing whether the inheritance of quantitative characters depends upon the same hereditary mechanism as does the inheritance of qualitative characters is to determine the linkage between these two kinds of characteristics.

There are relatively few published records of the linkage relations between quantitative characteristics and marker genes. Anderson (*1*) has tabulated the summary of work by Sax with weight of beans, of that by Lindstrom with weight of tomatoes and number of rows in corn, and of that by Smith with corolla size in tobacco. Twenty-two marker genes and six quantitative characters were investigated in these studies, and it is noted that linkage was found in all but possibly one of the cases. Hutchinson (*8*) found that lint index in Asiatic cotton is about 6 percent greater in plants with white corollas than in

plants with yellow corollas. Ware (*25*, *26*) reported that low lint-index values in upland cotton were associated with naked seed and that the lint indexes of okra-leaf segregates are lower than those of broad-leaf segregates in the F_2 population of a cross between okra-leaf and broad-leaf lines.

The demonstration of the relation between the green-lint factor and the lint-index values is rather simple. It can be accomplished by growing the F_2 and backcross generations of a cross between a white-lint, high lint-index cotton and a green-lint, low lint-index cotton and determining the significance of the difference between the mean lint-index values of the lint-color phenotypic classes. If the mean value for the white F_2 class is significantly higher than the corresponding value for the green F_2 class, evidence of the association of the green-lint factor and the lint-index values is provided.

The determination of the type of association that exists between a marker gene and a quantitative characteristic is not simple. It consists of determining to what extent the relation is due to linkage and to what extent it is due to pleiotropism.

If the relation is entirely the result of genetic linkage, it must be assumed that the lint index is affected by one or more factors that are located on the same chromosome as the green-lint gene. Such an assumption does not preclude or require the existence of lint-index factors in the other linkage groups. Except for the possibility of a very close linkage between major lint-index genes and the lint-color genes, each of the color phenotypes of the cross should segregate for the lint-index characteristic because of the free assortment of the chromosomes that do not carry basic lint-color genes and because of the chromosome segmental interchanges in the green-lint linkage group. Thus, it should be possible to obtain, in later generations, green-lint plants with lint indexes that, to some extent, approach the index of the white parent.

If the relation between the green lint and the lint index is due entirely to pleiotropism, it must be assumed that the gene that is responsible for the differences in lint color is also responsible for at least a part of the lint-index differences of the two parental lines. Grüneberg (*5*) has distinguished between "genuine pleiotropism," in which the gene produces its manifold effect through different primary effects, and "spurious pleiotropism," in which a single primary effect results finally in manifold effects. Anderson (*1*) has discussed pleiotropism as a hindrance to character recombination in the F_2, and Silow (*19*) has referred to the association of glabrousness and lintlessness in Asiatic cottons as being pleiotropic effects of the same gene. If lint index is affected to any extent by a pleiotropic effect of the basic gene for green lint, the variation of the lint-index values of the green-lint progenies would be restricted by the depressing effect of the green-lint gene; whereas the white-lint progenies of the cross would segregate for the lint-index character independently of the depressing effect of the basic lint-color gene.

MATERIALS AND METHODS

From the foregoing analysis, it follows that a study designed to determine the extent of the association of green lint and lint index, and the type of association, consists of making comparisons in regard

to the lint-index value between the phenotypes of hybrid populations, between the parental lines, and between the hybrid phenotypes and the parental lines.

Two varieties of upland cotton (*Gossypium hirsutum* L.) were used in these studies: Arkansas Green Lint (low lint index) and Half and Half white line (high lint index). The lines had been self-pollinated for several generations prior to the beginning of the study, and they were planted and inbred each season during the study to provide samples for comparison with the F_1, F_2, and F_3 phenotypic classes. In the F_1 generation the plants were backcrossed to each of the parental lines. Seeds from self-fertilized bolls for propagation and seed cotton from open-pollinated flowers for laboratory analyses were obtained from each F_1 plant.

The following year the two backcross and the F_2 populations were grown. Flowers on each of the F_2 plants were self-pollinated to provide seed for propagation of the F_3. Seed cotton from open-fertilized bolls was obtained from each backcross and F_2 plant for laboratory analyses.

During the next season, F_3 progenies from F_2 green, intermediate-green,[5] and white plants were grown. Samples of seed cotton were collected from each plant.

Laboratory analyses consisted of classifying each of the samples for lint color and of determining the lint-index value. There were three principal lint-color classifications: Green, intermediate green, and white. The green-lint phenotypes were divided into two subclasses (G_1 and G_2), and the intermediate-green-lint phenotypes into three subclasses (G_3, G_4, and G_5). The evidence that differences between subclasses within a phenotypic class are due to segregation and recombination of secondary genes is satisfactory.

For the purpose of studying the relation between the lint color and the abundance of lint produced on an individual seed, the seed index and lint percentage are not essential. It was revealed in preliminary analyses that, while the two parental lines differed significantly in regard to the seed index, the segregation in the F_2 plants for lint color was independent of the seed-index characteristic, and that the lint-percentage characteristic in these studies is associated with the lint colors in the same way as is the lint-index character.

RESULTS AND DISCUSSION

The frequency and the range and mean lint-index values of the lint-color phenotypes of the F_1 backcross (BC), F_2, and F_3 populations and of the corresponding parental lines are presented in table 1. Differences and significances of differences between values for lint index of comparable classes are given in table 2. The significances were determined by calculating the t values, according to a method suggested by Fisher (*3*) for cases in which no pairing of samples is possible and the number of samples is not the same for the two series of observations, and by comparing the obtained values with the t values required for the 5-percent and 1-percent levels of significance.

[5] Throughout this paper the term "intermediate green" refers to all segregates heterozygous for the basic factor pair *Lg*, *lg*, and is not confined to those heterozygotes with an intensity of pigmentation that is approximately half way between the intensities of the two parents.

TABLE 1.—*Frequency and range and mean lint-index values of the lint-color phenotypes of the F_1, F_2, BC_1, and F_3 populations and of the corresponding parental lines*

Generation	Phenotype	Frequency	Lint index	
			Range	Mean
			Grams	*Grams*
F_1	Intermediate green	37	4.43–5.97	5.13
Comparable P_1	Green	18	2.30–3.00	2.75
Do	White	19	[1] 4.83–9.20	7.45
BC ($F_1 \times$ G)	Intermediate green	24	2.94–4.94	4.05
BC ($F_1 \times$ W)	do	16	3.00–5.85	4.88
F_2	Green	113	1.95–4.74	3.01
F_2	Intermediate green	228	2.30–5.64	4.36
F_2	White	116	3.82–7.83	5.77
Comparable P_1	Green	16	2.13–3.06	2.63
Do	White	30	[2] 4.31–8.49	6.97
F_3 (from IG F_2)	Green	38	2.37–3.37	2.98
Do	Intermediate green	83	3.34–5.65	4.51
Do	White	26	3.89–7.87	5.71
F_3 (from G F_2)	Green	80	1.70–3.80	2.93
F_3 (from W F_2)	White	70	4.17–8.34	5.75
Comparable P_1	Green	8	2.44–3.06	2.66
Do	White	30	[2] 4.31–9.16	6.97

[1] Only 1 plant below 5.50 gm.
[2] Only 1 plant below 5.75 gm.

PARENTAL LINES

The original cross was made between green-lint and white-lint stocks with mean lint-index values of 2.75 and 7.45 gm. respectively. The parental green-lint stock was quite uniform, the range between the lowest and the highest value being 2.30 to 3.00 gm., or only 0.70 gm. The parental white-lint stock was considerably more variable, the range being 4.83 to 9.20 gm. Only one plant, however, had a lint-index value below 5.50 gm. The difference between the lint-index values of the two parental lines is highly significant, as shown in table 2.

TABLE 2.—*Differences and significance of differences in lint index of comparable classes of the F_1, F_2, BC_1, and F_3 populations and of the corresponding parental lines of the cross green lint $\times$ white lint*

Generation	Phenotypic class having—		Difference [1]
	Higher lint index	Lower lint index	
			Grams
F_1	Intermediate-green F_1	Parental green	2.38**
	Parental white	do	4.70**
	do	Intermediate-green F_1	2.32**
BC	Intermediate-green ($F_1 \times$ white)	Intermediate-green ($F_1 \times$ green)	.83**
F_2	White F_2	Green F_2	2.76**
	Intermediate-green F_2	do	1.35**
	White F_2	Intermediate-green F_2	1.41**
	Green F_2	Parental green	.38*
	Parental white	White F_2	1.20**
F_3	Intermediate-green F_3 from intermediate-green F_2.	Green F_3 from intermediate-green F_2	1.53**
	White F_3 from intermediate-green F_2	do	2.73**
	do	Intermediate-green F_3 from intermediate-green F_2.	1.20**
	Green F_3 from intermediate-green F_2	Parental green	.32
	Parental white	White F_3 from intermediate-green F_2	1.26**
	White F_3 from white F_2	Green F_3 from green F_2	2.82**
	Green F_3 from green F_2	Parental green	.27
	Parental white	White F_3 from white F_2	1.22**

[1] *Statistically significant, $P<0.05$. **Highly significant, $P<0.01$.

F_1 POPULATION

The 37 F_1 plants were classified as intermediate-green lint and had a mean lint-index value of 5.13 gm., which was intermediate between the lint-index values of the parents, indicating lack of dominance. The differences between the lint-index values of the green-lint parent and the F_1 and between the white-lint parent and the F_1 are each highly significant.

BACKCROSS POPULATIONS

Backcross populations consisted of the progenies of the $F_1 \times$ green-lint parent and of the $F_1 \times$ white-lint parent. Information regarding the relation of the lint-color and lint-index characteristics is afforded by the comparison of the lint-index values of the intermediate-green phenotypic classes of the two backcrosses. Theoretically, the two classes should be homologous in regard to the basic gene for the green-lint color. If the lint index is controlled by multiple factors, the $F_1 \times$ green genotypes should have a preponderance of lint-index genes from the green-lint parent, and the $F_1 \times$ white genotypes should have a preponderance of lint-index genes from the white-lint parent. In table 1 it is shown that the mean lint-index value is 4.05 gm., and the range 2.94 to 4.94 gm., for the intermediate-green class of the $F_1 \times$ green backcross, and 4.88 gm., with a range of 3.00 to 5.85 gm., for the intermediate-green class of the $F_1 \times$ white backcross. The mean difference between the two groups is 0.83 gm. and is highly significant.

There was considerable difference in the intensity of the green pigment in the two classes, the $F_1 \times$ green being much the more intense. Two alternative relationships are suggested: (1) The secondary genes that modify the expression of the green-lint factor in doing so modify the lint index, or (2) there is a linkage between the genes that affect lint color and the genes that affect the lint index.

F_2 POPULATION

The F_2 population consisted of 457 plants and segregated into three phenotypic classes, green, intermediate green, and white, approximately in a ratio of 1:2:1. The mean lint-index value of the green-lint class was 3.01 gm.; of the intermediate class, 4.36 gm.; and of the white class, 5.77 gm. There is considerable overlapping of lint-index values of the three phenotypes, the ranges for the green, intermediate green, and white classes being 1.95 to 4.74, 2.30 to 5.64, and 3.82 to 7.83 gm., respectively. The parental strains were continued, and comparisons with the F_2 segregations may be made. The mean lint-index value of the parental green-lint stock was 2.63 gm., with a range of 2.13 to 3.06 gm., and that of the parental white stock was 6.97 gm., with a range of 4.31 to 8.49 gm. It was noted that only one plant of the parental white line had a lint index value of less than 5.75 gm.

A study of the F_2 section of table 2 reveals that the differences in the lint-index values between the white and the green, the intermediate-green and the green, and the white and the intermediate-green classes of the F_2 were 2.76, 1.35, and 1.41 gm., respectively. Each of these differences is highly significant. The significant mean differences between the three phenotypic classes of the F_2 population verify

the association between the lint-index character and the gene affecting the green-lint character.

Some information regarding the association of the two characters is supplied by comparing the green-lint parental line with the green-lint F_2 population, having mean lint-index values of 2.63 and 3.01 gm., respectively, and by comparing the white lint parental line with the white-lint F_2 population, having mean lint-index values of 6.97 and 5.77 gm., respectively. The difference of 0.38 gm. between the green parent and the green F_2 is small and barely significant, whereas the difference of 1.20 gm. between the white parent and the white F_2 is highly significant, as shown in table 2. The Half and Half variety, used as the white-lint parent in this cross, is characterized by a higher lint-index value than most other upland varieties, as it is, no doubt, the product of 50 years of breeding and selection for a high-ginning outturn variety. Even if the suppressing effect of the green-lint gene were eliminated from the green-lint stock, this variety (Arkansas Green Lint) still would be expected to have a lower lint index than Half and Half.

On the basis of the results obtained in these studies, it is suggested that the green-lint parental line possesses low lint-index genes that segregate and recombine apparently independently of the basic green-lint gene. When these genes are combined with the white-lint character, replacing high lint-index allelomorphs, the lint index of the white F_2 is lower than that of the white parental line. Variation in the lint-index values of the white F_2 plants would be due, to some extent, to the segregation of the lint-index factors. At the same time, the mean lint-index value of the green F_2 should have been considerably higher than that of the parent green, since the high lint-index genes contributed by the white-lint parent would combine with the green-lint characteristic, replacing low lint-index allelomorphs of the green-lint parent. However, it is shown in table 2 that the difference between the lint-index value of the green F_2 and that of the parental green plants is very small and barely significant. It may be assumed that segregation took place as outlined, and that the expression of the new gene combination was suppressed to approximately the same level as the expression of the complex of the green parent, directly or indirectly, by the genes affecting the lint color.

F_3 POPULATIONS

The progeny of the intermediate-green F_2 plants segregated into three classes; green, intermediate green, and white, with mean lint-index values of 2.98, 4.51, and 5.71 gm., respectively. There was some overlapping of the intermediate-green and the white segregates. The difference between the mean lint-index values of the intermediate-green and green phenotypes is 1.53 gm.; between the white and green phenotypes, 2.73 gm.; and between the white and intermediate-green phenotypes, 1.20 gm. (table 2). Each of these differences is highly significant. The association between the lint-index character and the factor pair affecting the green-lint character is again demonstrated.

The parental lines were propagated in order that comparisons between the mean lint-index values of these lines and the respective F_3 phenotypes could be made. The difference between the parental green and green F_3 from the intermediate-green F_2 is only 0.32 gm.,

and the obtained t value of 1.57 is less than that required for a significance equivalent to odds of 19 to 1. The results are similar to those obtained in the F_2; and it is again suggested that, if high lint-index genes were acquired by recombination, their expression was prevented by the presence of some effect associated with the basic gene for green lint.

All of the F_3 green-lint segregates in the progeny of the F_2 intermediate-green plants were one grade less intense than were the plants of the parental line. Similar results were noted in the F_2 progeny. It is thus shown that a dilution of the green pigment is not always associated with an increase in the lint-index value. In the discussion of the backcross population it was stated that it is possible that the secondary genes that modify the expression of the green-lint factor in doing so modify the lint-index characteristic. The differences in intensity of the pigmentation of the $F_1 \times$ white and $F_1 \times$ green intermediate-green phenotypes were much greater than the differences between the parental green and the F_2 green or the parental green and the F_3 green. The intermediate-green phenotypes from the $F_1 \times$ white backcross were very dilute. In most plants the green pigmentation was confined to a small part of the sample of seed cotton, the remainder of the sample appearing to be completely devoid of green coloration. The intermediate-green phenotypes from the $F_1 \times$ green backcross produced seed cotton in which the green pigmentation was considerably more intense than the samples obtained from the intermediate-green F_1.

A comparison of the parental white line and the white-lint progeny of the intermediate-green-lint F_2 reveals a highly significant difference of 1.26 gm. and provides additional support to the deduction that the lint-index value of the white F_2 segregates can be decreased by factors obtained from the green-lint stocks in the absence of the basic green-lint gene.

In addition to the progenies of the F_2 intermediate-green-lint plants, progenies of the green-lint and white-lint F_2 plants were grown; in no progeny was there segregation for the basic lint-color factor pair. As shown in table 1, the mean lint-index values were 2.93 and 5.75 gm., respectively. By comparing the green F_3 from the green F_2 with the parental green (table 2), it was found that a difference in lint-index value of 0.27 gm. was not significant. The F_3 white-lint progeny of the F_2 white-lint plants was compared with the white-lint parental line, and a highly significant difference of 1.22 gm. in favor of the parental line was obtained.

EVIDENCE OF "SPURIOUS PLEIOTROPY"

Comparisons of the F_2 or F_3 green-lint phenotypes with the green-lint parent reveal little or no significant difference in mean lint-index value. Comparisons between the F_2 or F_3 white-lint phenotypes and the white-lint parental lines reveal that the F_2 and F_3 whites have a significantly lower lint-index value than does the parental white line. These associations suggest that in the segregation and recombination of lint-index genes low lint-index factors from the green-lint stock are combined with the white-lint gene, resulting in the F_2 white segregates having a lower mean lint index than the white parental line. In this segregation and recombination, it might logically be expected

that high lint-index factors from the white-lint stock would be combined with the green-lint gene. However, the data indicate that the green-lint gene suppresses the expression of the new complex to approximately the same lint-index level as the expression of the complex of the green-lint parental line.

While the multiple-factor theory of inheritance of quantitative characteristics has not been proved in these studies, no hypotheses were evolved that would, if verified, disprove the theory. If the lint-index value in white cottons is affected by a large number of factor pairs, it is quite probable that some of these genes are located in the green-lint linkage groups. However, this linkage relationship fails to account entirely for the association of the green-lint and lint-index characteristics. In the F_2 population it is relatively easy, merely by selecting green-lint phenotypes, to recover segregates that approach the parental lint-index combination of the green-lint stock. However, the recovery of combinations that approximate the white lint is much more difficult.

The evidence favors the theory that the green-lint and lint-index association is largely due to the pleiotropic effect of the basic green-lint gene. It appears that the effect is one of "spurious pleiotropy," the green-lint gene affecting the pigmentation in the fiber wall. In turn the pigmentation physiologically affects the lint index.

EFFECT OF GREEN PIGMENTATION

If the theory of "spurious pleiotropy" accounts for the association of the green-lint character and low index values, it must be assumed that the presence of the pigmentation affects the development of the fiber initials into normal fibers.

The diameter, wall thickness, estimated area, and maturity of fiber from the parental lines, the F_1 population, and the F_2 segregates were determined. The results are given in table 3.[6]

TABLE 3.—*Major diameter, minor diameter, ratio of circularity, wall thickness, and maturity of fibers from the parental lines, F_1 population, and F_2 phenotypes of the cross green lint × white lint*

Generation	Phenotype	Major diameter	Minor diameter	Ratio of circularity [1]	Wall thickness	Estimated area	Mature fibers
		μ	μ		μ	μ^2	*Percent*
P_1	Green	26.30	2.00	13.15	0.72	52.9	0
P_1	White	21.45	7.50	2.86	3.30	157.0	80
F_1	Intermediate green	22.17	5.79	3.83	2.42	129.0	40
F_2	Green	24.70	2.55	9.67	.85	62.2	0
F_2	Intermediate green	27.50	3.55	7.75	1.25	97.0	0
F_2	White	20.50	7.65	2.68	3.00	152.9	60

[1] Ratio of circularity = major diameter/minor diameter.

There was a marked difference in the cross-sectional characteristics of the green-lint and white-lint parental lines. The most outstanding difference was the wall thickness, the values being 0.72μ and 3.30μ for the green-lint and white-lint lines, respectively. The ratios of circularity for the two lines were 13.15 and 2.86, respectively.

[6] The values given in table 3 were determined by T. L. W. Bailey, Jr., associate cotton technologist, Agricultural Marketing Administration, U. S. Department of Agriculture, who also made the photomicrographs used in preparing figures 1 and 2.

The values for the F_1 intermediate green were intermediate between those for the parents, the wall thickness being 2.42μ and the circularity ratio being 3.83.

Although the F_2 intermediate green did not have as thick walls as the F_1 intermediate green, the association between the cross-section characteristics and the green pigmentation is obvious. The green-lint F_2 and white-lint F_2 samples were comparable to the respective parental types in regard to wall thickness and the ratio of circularity.

The values representing maturity were obtained by inspection of photomicrographs of the cross sections and comparison with a series of photomicrographs depicting different percentages of mature fibers, based upon extensive observations and actual counting of immature fibers according to the standard procedures proposed by Richardson, Bailey, and Conrad (*18*). The parent white and the F_2 white compared satisfactorily with average commercial cottons with respect to maturity. The F_2 was somewhat lower in maturity than the parent white, and the low lint percentage of certain F_2 white segregates may have been due to the higher percentage of immature fibers. Apparently the green possesses factors for immaturity other than those associated with green pigmentation. However, these factors are of less consequence than the green factor. The F_1 and F_2 intermediate-green plants produced fibers that were decidedly immature.

In figures 1 and 2, the differences in regard to diameter, ratio of circularity, and wall thickness are illustrated. Cross sections of fiber from the green-lint parent and the white-lint parent (fig. 1, *A* and *B*) show the small minor diameter and the thin wall of the fiber from the green-lint parent as compared with the larger minor diameter and thicker wall of the fiber from the white-lint parent. Cross sections of the F_1 intermediate-green fiber (fig. 1, *C*) show that the minor diameter and wall thickness are intermediate between those of the two parents.

Cross sections of fibers from the green-lint, intermediate-green-lint, and white-lint F_2 phenotypes (fig. 2, *A*, *B*, and *C*) show that the fibers from the recovered green and white phenotypes compare closely with the respective parental lines in regard to minor diameter and wall thickness and that the fibers from the F_2 intermediate-green phenotype are intermediate between the green and the white phenotype.

The data shown in table 3 and in figures 1 and 2 indicate that the presence of the green pigmentation in the cell wall results in immature fibers with thin walls and relatively small minor diameters. The individual fibers of the green segregates weigh less than those of the white segregates, and consequently the lint index for the green fiber is less.

SUMMARY AND CONCLUSIONS

Crosses were made between two strains (Arkansas Green Lint and Half and Half white lint) of upland cotton (*Gossypium hirsutum* L.), characterized respectively by green lint, low lint index, and white lint, high lint index.

The F_1 was intermediate green with a mean lint-index value that was intermediate between those of the two parents.

In the backcross generations, satisfactory 1:1 ratios of green to intermediate green or intermediate green to white were obtained. In

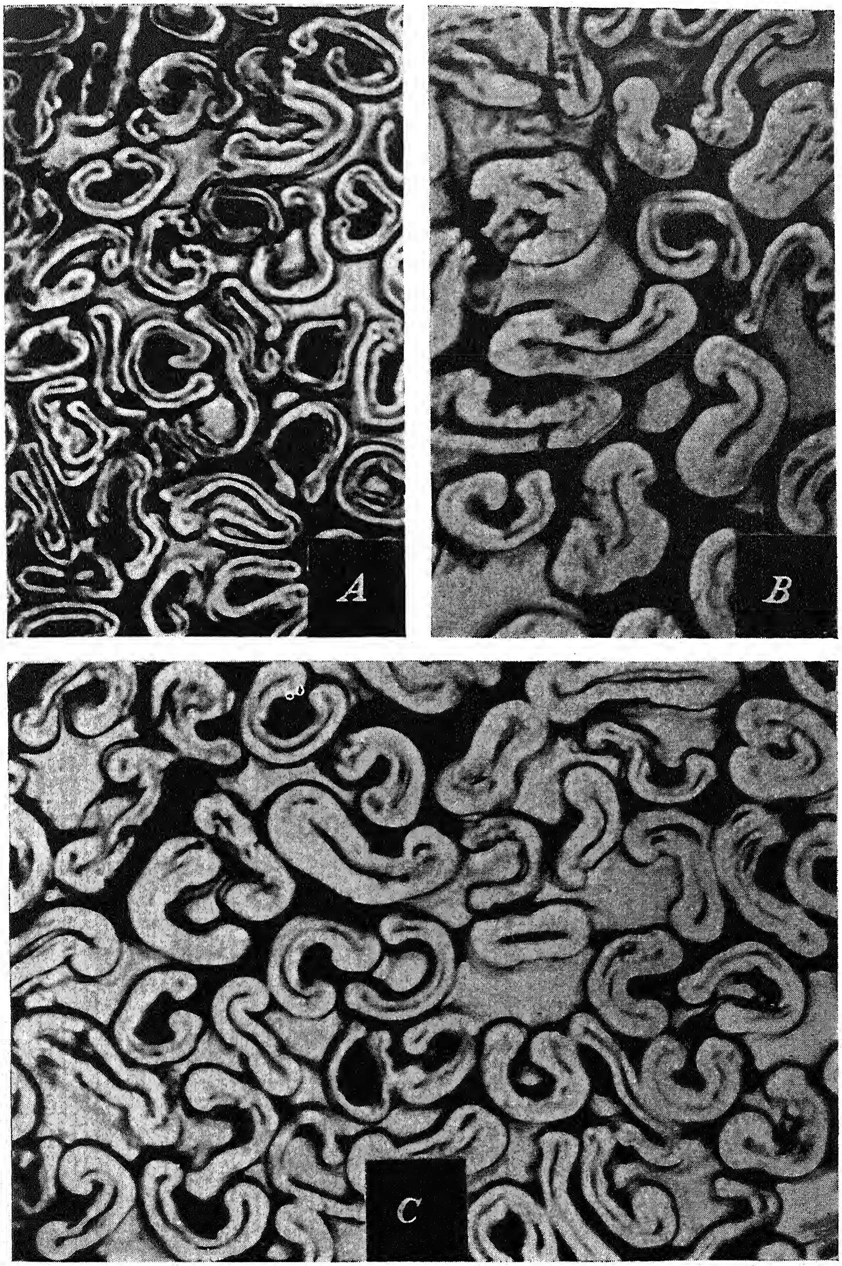

FIGURE 1.—Cross sections of cotton fibers from (*A*) green-lint parent, (*B*) white-lint parent, (*C*) F_1 intermediate-green lint of green × white cross. × about 1,000.

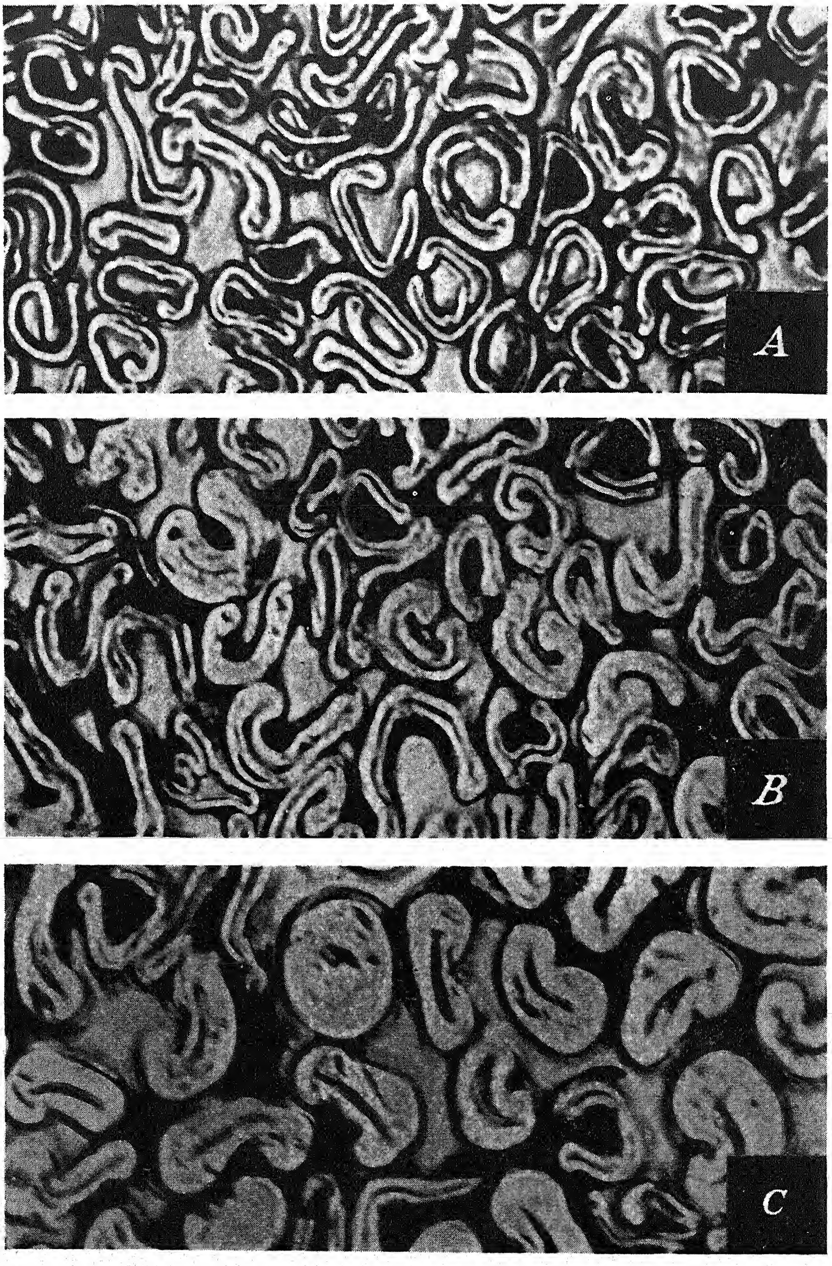

FIGURE 2.—Cross sections of cotton fibers from F_2 phenotypes of the cross green lint × white lint: *A*, Green-lint segregate; *B*, intermediate-green-lint segregate; *C*, white-lint segregate. × about 1,000.

the F_2 generation, satisfactory 1 : 2 : 1 ratios of green, intermediate green, and white were obtained. The previous conclusion that the characteristic is controlled by one genetic factor pair is confirmed.

In the analysis of samples from the backcross, F_2, and F_3 phenotypes it was shown that green lint and low lint index are very closely associated. Linkage fails to account for the association. When high lint-index factors from the white-lint stock are combined with the green-lint gene, the green-lint gene suppresses the expression of the lint-index genes. The green-lint and lint-index association is largely due to the pleiotropic effect of the basic green-lint gene.

The effect appears to be one of "spurious pleiotropy," the green-lint gene affecting the pigmentation in the fiber wall. In turn, the pigmentation prevents the development of the fibers. The result is that green fibers have thin walls and small minor diameters and consequently very low lint-index values.

It is indicated that green-lint types with a lint index approximating that of commercial white cottons cannot be developed through breeding.

Perhaps the "spurious pleiotropic" effects of factors may account for many cases of so-called antagonism between characteristics of domesticated plant species.

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HERITABLE RELATION OF WAX CONTENT AND GREEN PIGMENTATION OF LINT IN UPLAND COTTON[1]

By Carl M. Conrad, formerly *senior cotton technologist, Agricultural Marketing Administration,* and J. Winston Neely, *geneticist, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

The wax content of cotton lint has been the subject of a number of investigations. Lecomber and Probert (*8*)[3] studied cottons originating in different parts of the world in order to determine the extent to which their origins could be identified by the content and properties of wax. It was found that cottons could not be identified in this way. Fargher and Higginbotham (*6*) studied scoured cottons with reference to the percentage losses sustained and the amount of certain noncellulose constituents, including waxes, retained after various kiering treatments. Ahmad and Sen (*1*) were interested in the possible influence of wax content on the classer's judgment of "silkiness" or "harshness." Fargher and Probert (*4*), Clifford and Probert (*2*), and Fargher and Higginbotham (*5*) have reported that the different components of the wax from the lint in American and Egyptian cottons are highly complex mixtures of various classes of waxy substances.

As early as 1911, Knecht (*7*) reported that Egyptian raw cotton from which the wax had been removed was difficult to handle in the drawing and spinning processes. Excessive amounts of waste, irregular results, and a tendency for the fiber to adhere to the rollers were encountered. In spinning finer count yarns, excessive breakage, which was also observed in the weaving processes, occurred. Knecht suggested that benefits in the manufacturing process and in the strength of the material might result from supplementing the low natural wax content of ordinary cotton fiber with additional oily or waxy substances. This has been done occasionally by spraying mineral oils and other substances on the fiber at different stages in the progress of manufacture.

Conrad (*3*) has shown that whereas the wax content of most cotton lint varies within the range of from 0.4 to 0.7 percent, that of Arkansas Green Lint cotton, based upon the dry weight, reached the high value of 17 percent. This finding suggested the possibility of studying the influence of a high natural wax content on the spinning quality of cotton.

The Arkansas Green Lint cotton is not suitable for commercial production because of its small yield and low ginning outturn. Neely (*9*) has shown that the green pigmentation and low ginning outturn are "spurious pleiotropic" effects of the same gene and that it is very unlikely that green-lint strains with lint percentages approximating

[1] Received for publication June 16, 1942. Cooperative investigations between the Agricultural Marketing Administration and the Bureau of Plant Industry, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station. The senior author is now with the Southern Regional Research Laboratory, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, New Orleans, La. When the investigation was made this work was a part of the Agricultural Marketing Service. On February 23, 1942, that Service was merged with the Surplus Marketing Administration and the Commodity Exchange Administration to form the Agricultural Marketing Administration.

[2] The authors wish to acknowledge their indebtedness to Dr. J. O. Ware, senior agronomist, Bureau of Plant Industry, who made helpful suggestions in regard to the manuscript, and to Meyer D. Silverman, scientific aide, Agricultural Marketing Administration, who made the wax determinations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 312.

the commercial white cottons can be developed through breeding. In many cases the green-lint cotton would not be suitable for commercial production because of its color and fiber shortness. Since no other cotton is known that has the high wax content of the green-lint strains, interest has been manifested in the possibility of combining, through hybridization and subsequent selection, the high wax content of the green-lint strains with the lint color and other desirable characteristics of the commercial white-lint strains. Thus, information regarding the type of heritable relation that exists between the wax content and the green-lint character is of considerable importance. It was the purpose of this investigation to study these relations.

MATERIAL AND METHODS

The samples employed in these studies were collected at Stoneville, Miss., during the 1938 and 1939 crop season and were used in studies to determine the relation of green lint to lint index in upland cotton. Two strains of cotton were used in these studies: Arkansas Green Lint (high wax content) and Half and Half white lint (low wax content). These strains were self-pollinated for several generations prior to the beginning of the study.

The methods employed for selecting parent plants and for self- and cross-pollination have been described by Ware (*10*). The procedure followed in obtaining samples from parental lines and of F_1, backcross, F_2, and F_3 segregations and in classifying the samples, as well as the mode of inheritance of the green-lint characteristics, have been outlined by Neely (*9*).

Lint samples of the 1938 growth from all plants of a given lint-color classification and parental line or progeny were composited and thoroughly mixed for wax-determination samples.[4] Because of the large number of plants in the 1939 growth, only about half of the plants of a given lint-color classification and progeny were composited for each determination.

After compositing and thoroughly mixing, duplicate $10 \pm .01$-gm. samples of lint were weighed out and placed, without grinding, in the extraction compartments of 50 by 250 mm. Soxhlet extractors, provided with 300-ml. distillation flasks. Thimbles were not used. The material was compacted so that all fiber would be covered with the condensed liquid before the latter siphoned over. Then, 250 ml. of 95-percent alcohol was poured into the cotton; a portion siphoned over and collected in the flask below. The condenser was attached, the water turned on, and the extraction continued for 6 hours from the time of boiling. At the end of this period the flame under each flask was turned out at the moment (within 1 to 5 minutes) that the alcoholic extract remaining in the flask amounted to about 100 ml.

The amount of wax contained in the alcoholic solution was determined by a method to be described elsewhere by Conrad.[5] Briefly, it consists of the transfer of the hot alcoholic solution to 100 ml. of chloroform in a 1,000-ml. separatory funnel, the addition of sufficient distilled water to cause separation, and the removal of the chloroform layer from the bottom. The aqueous layer is then washed three times

[4] Although, from the genetical standpoint, it would be desirable to determine, separately, the wax content of the lint from each plant of the progenies, the amount of work involved was prohibitive for the amount of time that could be allotted.

[5] CONRAD, C. M. A NEW EXTRACTION METHOD FOR THE DETERMINATION OF TOTAL WAX IN COTTON FIBER. (In preparation.)

with 50-ml. portions of chloroform, after which the combined chloroform extracts are washed once with distilled water. The sugars and other water and alcohol-soluble substances pass more or less completely to the water layer, while the waxy substances are retained in the chloroform layer. The chloroform-soluble fraction is then evaporated and dried in a tared weighing bottle at 105° C., weighings being made at 30-minute intervals until the loss is not more than 1 mg.

Separate determinations of moisture were made, and the wax content was computed to the oven-dried weight.

RESULTS

The percentages of wax of the lint-color phenotypes of the F_1, backcross, F_2, and F_3 populations and of the corresponding parental lines from the 1938 and 1939 growths are given in tables 1 and 2, respectively. The percentage of wax in each case represents the mean of two or more closely agreeing values.

TABLE 1.—*Wax content of lint-color phenotypes of F_1 and first-generation backcross progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1938 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_2	1	P_1 parents	0.63		15.04
BC	1	$F_1\times$ green-lint parent		3.42	13.92
BC	1	$F_1\times$ white-lint parent	.60	2.11	
F_2	1	F_1 (white $\times$ green)	.54	3.96	12.32
P_1	2 and 3	Parents of F_1	[1] .56		13.12
F_1	2 and 3	Green- $\times$ white-lint parents		[2] 2.17	

[1] Lint from only 1 plant represented.
[2] Lint from 2 families, 2 and 3, combined.

TABLE 2.—*Wax content of lint-color phenotypes of first-generation backcross, second-generation backcross, F_2, and F_3 progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1939 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_3	1	Family 1 parents	0.48		12.64
BC_2	1	Intermediate BC on green	.53	2.84	12.53
BC_2	1	Intermediate BC on white	.58	2.36	12.13
F_3	1	Green F_2 phenotype			12.90
F_3	1	Intermediate F_2 phenotype	.56	2.26	11.36
F_3	1	White F_2 phenotype	.66		
P_2	2	Family 2 parents	.63		12.82
BC	2	$F_1\times$white-lint parent	.59	1.95	
F_2	2	F_1 (white$\times$green)	.51	2.40	12.42
P_2	3	Family 3 parents	.61		12.82
BC	3	$F_1\times$white-lint parent	.55	1.92	
F_2	3	F_1 (white$\times$green)	.54	2.52	12.12

PARENTAL LINES

The original cross was made between white-lint and green-lint stocks with wax percentages of 0.63 and 15.04, respectively. Inbred progenies of the parental lines were grown each season, and very little variation in the wax content was found. The range was from 0.48 to 0.63 percent for the white-lint parental line and from 12.64 to 15.04 percent for the green-lint line. The difference in the wax content of the two stocks is striking.

THE F_1 POPULATION

The F_1 plants were classified as intermediate green, and the wax content was found to be 2.17 percent, as shown in table 1. Thus, there is not complete dominance in regard to the wax content, but the F_1 value is nearer that of the low-wax-content parent.

THE BACKCROSS POPULATION

Backcross populations consisted of the progenies of the $F_1\times$green-lint parent and of the $F_1\times$white-lint parent crosses. Information regarding the relationship of the lint-color and wax-content characteristics is afforded by the comparison of the wax percentages of the intermediate-green phenotypic classes of the two backcrosses. Theoretically, the two classes should be homologous for the green-lint color basic-factor pair. The $F_1\times$green genotypes should have a preponderance of wax-content genes from the green-lint parent and the $F_1\times$white genotypes should have a preponderance of wax-content genes from the white-lint parent, if the wax content is controlled by multiple factors. It is shown in table 1 that the mean wax content is 3.42 percent for the intermediate-green class of the $F_1\times$green backcross and 2.11 percent for the intermediate-green class of the $F_1\times$white backcross, or a difference of 1.31 percent.

The $F_1\times$green intermediate-green progenies were considerably more intensely green than were the $F_1\times$white intermediate-green progenies. It is indicated that the secondary genes that modify the expression of the green-lint factor also modify the wax content.

The wax content of the intermediate-green segregates of the backcross $F_1\times$white of families 2 and 3 is 1.95 and 1.92 percent, respectively. The white-lint segregates of the two families have average wax percentages of 0.59 and 0.54 percent.

The progeny of the intermediate-green phenotypes of the backcrosses segregated into 1 white: 2 intermediate green: 1 green. The wax content of these classes was 0.53, 2.84, and 12.53, percent, respectively, for the backcross to the green parent and 0.58, 2.36, and 12.13 percent, respectively, for the backcross to the white parent. The wax content of the comparable white-lint and green-lint parental lines is 0.48 and 12.64 percent, respectively. The difference between the wax content of the white and green segregates and the corresponding parental type is apparently of no consequence. The wax-percentage differences between the three phenotypes of the second backcross generations verify the association between the wax-content character and the gene affecting the green-lint character.

THE F_2 POPULATIONS

The F_2 populations, consisting of one family in 1938 and two families in 1939, were grown. Segregation for the lint-color character in each family corresponded satisfactorily to the expected 1:2:1 ratio

of white, intermediate green, and green phenotypes. The association of high wax content and green lint is shown. The wax percentages for the white, intermediate-green, and green phenotypes, as shown in tables 1 and 2 for family 1, were 0.54, 3.96, and 12.32, respectively; for family 2, 0.51, 2.40, and 12.42; and for family 3, 0.54, 2.52, and 12.12. Comparable white-lint parental lines for the three families had wax percentages of 0.63, 0.63, and 0.61, respectively; and comparable green-lint parental lines for the three families had wax percentages of 15.04, 12.82, and 12.82, respectively. It is possible that the wax content of the F_2 green-lint segregates was significantly lower than that of the green-lint parental lines. Again it is indicated that the secondary genes that modify the expression of the green-lint factor pair also modify the wax content, since the F_2 green-lint segregates were less intensely green than the green-lint parental lines.

THE F_3 POPULATIONS

The progeny of the intermediate green F_2 plants segregated into three classes: green, intermediate green, and white, with mean wax percentages of 0.56, 2.26, and 11.36, respectively. The association between the wax content and green-lint pigmentation is again demonstrated.

The parental lines were propagated in order that comparisons between the wax percentages of these lines and the respective F_3 phenotypes could be made. The difference between the parental white and the F_3 white from the F_2 heterozygote is 0.08 percent and is probably of no consequence. Similarly, the difference between the parental green and the F_3 green from the F_2 intermediate green is rather small in comparison with the difference between the two phenotypes.

The F_3 white progeny from the F_2 white phenotypes had a wax content of 0.66 percent as compared with a wax content of 0.48 percent for the parental white. There is some indication that small differences in wax content which are not associated with differences in lint pigmentation may occur. The difference between the wax content of the F_3 green progeny from th F_2 green phenotypes and the green-lint parental line is small and probably of no significance.

SUMMARY AND CONCLUSIONS

The total wax content of lint from Half and Half white lint and Arkansas Green Lint upland cottons and of the F_1, backcross, F_2, and F_3 populations of crosses between the two strains was determined. The samples represent two consecutive crop years and three sets of families representing three different crosses. The wax content of the white-lint parent varied within the low limits of 0.48 to 0.63 percent and that of the green-lint parent between the much higher limits of 12.64 to 15.04 percent.

The F_1 was intermediate green with a mean wax content between that of the two parents but closer to that of the white parent.

In the analyses of samples from the backcross, F_2, and F_3 phenotypes, it was shown that the green lint and high wax content were closely associated.

While there is no evidence against the relationship being one of genetical linkage, it is indicated that the relationship is probably a physiological one and that the genetic factor that affects the green

pigmentation also affects the wax content. Previous work has shown that the presence of green pigmentation in the fiber cell wall is associated with a suppressed development of fiber initials into mature fibers. Just what is the interrelationship of cause and effect between high wax content, green pigmentation, and suppressed development of fiber wall remains to be clarified.

The results are not conclusive, but it is indicated that there is little likelihood of combining the high wax content of the green-lint strain with the white lint of commercial cotton. It is possible that the genetic behavior in crosses between other green-lint strains and the same or other white-lint varieties would be different. These studies are being extended to include such crosses.

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ASCORBIC ACID CONTENT OF STRAINS OF SNAP BEANS [1]

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INTRODUCTION

In 1936 a program of breeding hardy, productive, high-quality snap beans for the southern United States was undertaken at the United States Regional Vegetable Breeding Laboratory, Charleston, S. C. Although the desirability of incorporating high ascorbic acid content, as well as other desirable qualities, in the lines of snap beans being developed was evident, satisfactory methods for the determination of this vitamin on large numbers of samples were not available when the work was initiated. Since then, however, rapid and accurate methods have been devised for the determination of ascorbic acid; these have made possible investigations in the plant-breeding program that were not feasible a few years ago. The subjects in the present study were relatively finished varieties of snap beans, some of which are pure lines, but the techniques used herein are also applicable to studies of the ascorbic acid content of segregating lines.

MATERIALS AND METHODS

In 1940, 46 strains of beans (*Phaseolus vulgaris* L.) derived from crosses of U. S. No. 5 Refugee (or closely related lines) with Stringless Black Valentine, Bountiful, and Brittle Wax were released to the 13 collaborating Southeastern States for trial. Some of these strains when released were quite uniform; others were segregating for seed color, pod types, and other factors. While these beans were being grown by some of the collaborating States, they were also being grown at Charleston, S. C. For the crops of 1940, rapid chemical methods for the determination of ascorbic acid content were not available. However, preliminary work by Morell (*9*) [2] resulted in an improvement for use here of Bessey's (*1*) modification of Mindlin and Butler's (*8*) method. The crops of 1941 were analyzed for ascorbic acid content at each of four harvests of each crop.

The strains were arranged in a 7 by 7 lattice square with 4 series (replicates), according to a method of Weiss and Cox (*13*). For convenience the strains were numbered 1 to 49, with 9, 48, and 49 representing the commercial varieties Brittle Wax, Bountiful, and Stringless Black Valentine, respectively, and the other numbers, which are preceded by VBL,[3] the 46 crosses previously mentioned. All strains had previously been observed to mature for market within a day or two of each other under Charleston conditions. Plots were single rows 3 feet apart and 32 feet long. Locally grown seed of the

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[2] Italic numbers in parentheses refer to Literature Cited, p. 324.
[3] VBL indicates a strain originated by the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

46 strains originated by the United States Regional Vegetable Breeding Laboratory was used throughout the experiment, but western-grown seed of the 3 commercial varieties was used. For the most part good stands were obtained.

Spring and fall crops of pods were each harvested four times. A complete harvest of pods never required more than 4 hours. Only pods at market or canning stages were included, thus eliminating variability due to immaturity or overmaturity of pods. Samples were placed in bags, stored at 36° F. within 30 minutes after picking, and analyzed within 48 hours. Twenty-five grams of unselected whole pods was used for analysis; broken and damaged pods were excluded.

Two pickings of leaves were made of each crop—the first a few days after the second picking of pods and the second a few days after the fourth picking of pods. Leaf harvests were begun at about 8:30 a. m. and were completed in 2 hours. Samples of 25 gm. were picked, and 10 gm. was used for analysis.

The following procedure, developed by Morell (*9*), was used in analyzing the samples. After the stems and tips had been removed, the pods were broken into four parts. The petioles were removed from the leaves. A suitable sample of pods or leaves, weighed to the nearest 0.1 gm., was added to 100 ml. of 3-percent metaphosphoric acid in a blender container and mixed at high speed for 2 minutes. This produced a fine suspension, which was filtered through a No. 12 Whatman paper. A convenient aliquot of the filtrate was transferred to a 50-ml. volumetric flask and adjusted to pH 3.6 by the addition of 0.25 ml. of a sodium citrate buffer per milliliter of aliquot. The mixture was then made to volume with a citrate-phosphate buffer. The pH of the final solution was maintained at 3.6 ± 0.1. A 5-ml. portion of this solution was transferred to an absorption tube containing 5 ml. of the indicator (sodium 2,6-dichlorobenzenoneindophenol), and the photometric reading was made with an Evelyn photoelectric colorimeter with filter No. 520. Blank readings were obtained with 5 ml. of the citrate-phosphate buffer. The analyses were made in groups of 24, and approximately 100 determinations were completed in a day. The percentage of water in the plant tissue, which must be taken into account in calculating the ascorbic acid content, was determined by drying weighed samples in an electric oven at 103° C. for 18 hours.

The manganese in the soil was determined by the ammonium persulfate method (*12, p. 305*); an Evelyn photoelectric colorimeter with a No. 540 filter was used. Soil samples were taken with a soil auger to a depth of 14 inches from three places in each plot. The samples were taken immediately after the fourth harvest of pods in the spring.

During the period from planting to the first spring harvest in 1941 there occurred one of the severest droughts on record for this area. The beans were irrigated three times, but the heat was so intense that at times many of the plants drooped because they were not able to take up moisture as fast as needed. Many local commercial, nonirrigated bean plantings were abandoned, but the beans at the Vegetable Breeding Laboratory produced a normal crop, with only very slight nonrecovery from the drooping observed.

During the fall of 1941 the temperatures were much above normal for this area until about the time of the third harvest of pods. The fourth harvest of pods and the second picking of leaves occurred after the temperature had dropped to 28° F. for a short while. Only pods and leaves showing no frost damage were used in the chemical analyses. This planting was irrigated twice, but other beans grown without irrigation gave fair yields.

RESULTS

PRELIMINARY AND INCIDENTAL INVESTIGATIONS [4]

A study was made to determine whether there was any relation between stage of maturity and ascorbic acid content. For this work six varieties representing both small- and large-podded types were used. Within each strain small size represented a relatively immature pod, large size a slightly overmature one, and medium the size ordinarily harvested for canning or marketing. Equal numbers of records were not available for each strain. There were no significant differences between large, medium, and small pods within a strain. The ascorbic acid content for 58 pods of each size averaged 17.2, 16.9, and 17.6 mg. per 100 gm., respectively.

The effect on ascorbic acid content of storage at 36° F. and at 98 to 100 percent relative humidity was studied partly in combination with the stage-of-maturity experiment and partly on other materials. There was a slight but nonsignificant increase in ascorbic acid content up to 72 hours, but at 96 hours there was a highly significant decrease of 6.94 mg., or 38 percent, from that of the 72-hour period (table 1).

TABLE 1.—*Effects of storage at 36° and 70° F. on ascorbic acid content of snap beans*

Average storage temperature (° F.)	Ascorbic acid content per 100 gm. (fresh-weight basis) after storing for the number of hours specified [1]				
	None [2]	24–48	72	96	168
	Milligram	*Milligram*	*Milligram*	*Milligram*	*Milligram*
36 [3]	17.87	17.46	18.38	11.44**	
70 [4]	16.25	15.04	10.98**		9.31**

[1] ** indicates that content was highly significantly different from that of samples that were analyzed immediately after picking (column 2).
[2] The difference in entries in this column is due to the difference in the varieties tested in the 2 groups.
[3] 15 determinations at each period.
[4] 30 determinations at each period.

In another experiment the effects of storage at room temperature (70° F.) on ascorbic acid content were studied for various lengths of time up to 1 week. There was a slight decrease at 48 hours after harvest, and a sharp and significant one at 72 hours. At 168 hours there was a further decrease to somewhat more than half the initial amount, but the content was not significantly lower than at 72 hours (table 1). The storage room was rather favorable for holding the beans in good condition, since it was cool and well ventilated and the beans were not exposed to sunlight. In this experiment relative humidity was not recorded.

[4] Parts of the preliminary investigations were carried out by S. A. Morell, formerly of the Division Fruit and Vegetable Crops and Diseases.

A study was undertaken in the fall of 1941 to determine the distribution of ascorbic acid in various parts of the plant. Plants were selected from rows from which no pods or leaves had been harvested, and determinations were made on roots, pods, leaves, and stems. The large, sparsely leaved variety Stringless Black Valentine and the small, abundantly leaved strain VBL 19 were used. The roots were found to contain about half as much ascorbic acid as the pods, the leaves four to five times as much as the pods, and the stems as much as or nearly twice as much as the pods, depending upon the variety (table 2).

TABLE 2.—*Ascorbic acid content of various parts of bean plants*

[Average of 3 determinations per variety for each part]

Variety or strain	Ascorbic acid content per 100 grams (fresh-weight basis) of—			
	Roots	Pods	Leaves	Stems
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Stringless Black Valentine	10.62	26.38	101.19	27.93
VBL 19	9.11	19.22	104.71	37.74
Average	9.87	22.80	102.95	32.84

A further study was made to determine the range of ascorbic acid content of a widely divergent group of beans. The lowest content per 100 gm. (8.13 mg.) was found in the pods of a promising hybrid, while the highest content (36.55) was found in those of the field bean Idaho No. 1 Mosaic Resistant Great Northern. In general, tender, high-quality strains or varieties, such as Blue Lake (12.23), U. S. No. 5 Refugee (19.40), and Kentucky Wonder (15.99), were low in ascorbic acid content, while the more fibrous strains or varieties had a higher content. An exception was the fibrous Sure Crop Wax, which was low in ascorbic acid content (13.83).

MAIN INVESTIGATIONS

The average ascorbic acid content of the 49 strains or varieties grown in the spring ranged from 19.1 to 28.7 mg. per 100 gm. per strain (table 3), while the values for the same strains grown in the fall were from 16.2 to 26.4. The averages for fall and spring combined ranged from 17.7 to 27.5 mg. The highest ranking variety (No. 48) was Bountiful; the lowest was the hybrid strain VBL 6 (table 3).

There was a slight but highly significant decline in the ascorbic acid content of the spring crop of pods from the first two pickings to the third and fourth (table 4), but in the fall the first picking had an average of less than half that of the first spring picking (12.16 vs. 24.41 mg. per 100 gm.), and the difference is highly significant. The ascorbic acid content of the pods from the second fall picking was significantly below the average for the corresponding spring picking. The third fall picking was not significantly different from the third spring picking, but the fourth was highly significantly above any of the other seven pickings. The coefficients of variability were 13.34 percent for the spring and 16.80 percent for the fall pickings. The average ascorbic acid content was 23.39 mg. per 100 gm. for spring pickings, and none of the four series (replicates) diverged more than 4.96 percent

TABLE 3.—*Ascorbic acid content of pods and leaves of 49 strains or varieties of snap beans, Charleston, S. C., 1941*

Cross or variety	Pods						Leaves					
	Spring		Fall		Both		Spring		Fall		Both	
	Ascorbic acid content per 100 gm. (fresh-weight [1] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight [1] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight [2] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight [3] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight [3] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight [1] basis)	Rank
	Milligrams		*Milligrams*		*Milligrams*		*Milligrams*		*Milligrams*		*Milligrams*	
VBL 1	20.0	46	17.4	46	18.7	46	121.6	26	144.8	43	133.2	40
VBL 2	21.9	35	18.5	40	20.2	41	109.4	46	160.3	22	134.9	36
VBL 3	21.3	38	18.3	41	19.8	42	100.6	49	153.0	35	126.8	46
VBL 4	21.4	37	17.7	44	19.5	43	109.8	44	155.6	30	132.7	41
VBL 5	20.3	43	21.6	14	20.9	34	107.5	48	159.9	23	133.7	39
VBL 6	19.1	49	16.2	49	17.7	49	108.4	47	142.5	46	125.4	47
VBL 7	19.4	48	17.6	45	18.5	47	120.8	28	150.2	37	135.5	34
VBL 8	23.1	29	20.6	20	21.9	24	125.6	17	147.9	40	136.8	32
Brittle Wax (No. 9)	25.6	9	21.3	16	23.5	11	132.8	4	155.2	34	144.0	17
VBL 10	23.5	25	22.9	7	23.2	13	118.2	35	164.7	15	141.4	20
VBL 11	22.2	33	22.8	8	22.5	20	121.3	27	157.8	26	139.6	25
VBL 12	25.2	10	21.3	15	23.3	12	124.5	21	155.5	31	140.0	24
VBL 13	24.6	16	23.7	5	24.1	8	112.9	43	161.4	19	137.2	31
VBL 14	23.2	28	19.1	33	21.1	30	129.0	8	182.6	2	155.8	2
VBL 15	20.1	44	21.9	12	21.0	33	123.8	22	164.6	16	144.2	16
VBL 16	20.0	45	16.4	48	18.2	48	117.4	37	147.4	41	132.4	43
VBL 17	27.9	3	20.3	21	24.1	7	122.9	24	169.3	8	146.1	13
VBL 18	21.2	41	19.9	26	20.5	37	120.2	29	155.3	33	137.7	29
VBL 19	26.6	5	19.2	32	22.9	15	109.8	45	170.5	7	140.1	23
VBL 20	23.3	27	20.2	23	21.8	25	118.7	33	149.3	39	134.0	38
VBL 21	23.6	23	19.8	29	21.7	26	126.4	15	161.0	20	143.7	18
VBL 22	22.1	34	18.8	38	20.4	38	116.0	38	147.2	42	131.6	45
VBL 23	22.6	31	18.2	42	20.4	40	123.6	23	162.9	18	143.2	19
VBL 24	25.1	11	20.3	22	22.7	19	119.0	32	156.7	27	137.8	28
VBL 25	19.6	47	18.1	43	18.8	45	119.6	30	144.3	45	132.0	44
VBL 26	23.6	24	18.6	39	21.1	31	117.4	36	150.9	36	134.2	37
VBL 27	24.1	20	19.8	28	21.9	23	113.3	40	156.6	29	134.9	35
VBL 28	24.8	15	24.4	4	24.6	4	127.2	11	155.5	32	141.3	21
VBL 29	25.0	13	22.6	9	23.8	9	113.1	41	137.2	48	125.2	48
VBL 30	20.4	42	21.0	19	20.7	35	113.1	42	134.1	49	123.6	49
VBL 31	25.0	12	23.2	6	24.1	6	122.4	25	184.3	1	153.4	3
VBL 32	21.5	36	19.9	27	20.7	36	114.9	39	159.6	24	137.3	30
VBL 33	26.3	6	19.7	30	23.0	14	129.4	7	165.0	14	147.2	11
VBL 34	24.2	19	18.9	37	21.6	27	125.4	18	163.2	17	144.3	15
VBL 35	24.0	21	20.1	24	22.0	22	131.2	5	150.0	38	140.6	22
VBL 36	23.9	22	19.0	35	21.4	28	134.2	3	161.0	21	147.6	9
VBL 37	21.2	39	19.7	31	20.4	39	127.6	10	166.2	12	146.9	12
VBL 38	24.6	17	21.0	18	22.8	17	129.5	6	173.2	4	151.4	5
VBL 39	25.7	8	20.0	25	22.9	16	118.5	34	157.9	25	138.2	26
VBL 40	22.5	32	21.7	13	22.1	21	125.7	16	169.1	9	147.4	10
VBL 41	27.1	4	22.0	11	24.6	5	127.6	9	168.8	10	148.2	8
VBL 42	24.4	18	21.2	17	22.8	18	134.7	2	168.5	11	151.6	4
VBL 43	23.1	30	19.1	34	21.1	32	126.5	14	172.1	5	149.3	6
VBL 44	23.4	26	19.0	36	21.2	29	135.8	1	179.8	3	157.8	1
VBL 45	24.9	14	22.4	10	23.7	10	125.0	19	166.0	13	145.5	14
VBL 46	28.2	2	26.4	1	27.3	2	124.9	20	139.9	47	132.4	42
VBL 47	21.2	40	17.4	47	19.3	44	127.2	12	144.6	44	135.9	33
Bountiful (No. 48)	28.7	1	26.3	2	27.5	1	126.8	13	170.7	6	148.8	7
Stringless Black Valentine (No. 49)	25.8	7	25.4	3	25.6	3	119.2	31	156.6	28	137.9	27
Average	23.39	------	20.42	------	21.91	------	121.44	------	158.58	------	140.0	------
Significant difference:												
5-percent point [4]	2.53	------	2.78	------	1.88	------	14.69	------	24.28	------	13.45	------
1-percent point	3.35	------	3.64	------	2.49	------	19.45	------	32.15	------	18.67	------

[1] Average of 4 harvests (16 determinations).
[2] Average of 8 harvests (32 determinations).
[3] Average of 2 harvests (8 determinations).
[4] Significant difference for strain means.

from this amount; in the fall the average was 20.42 mg., and none of the series diverged more than 5.53 percent. If the first picking in the fall is excluded, the average for fall is 23.17, almost identical with the spring value.

In both spring and fall there was a decline in ascorbic acid content of the leaves from the first picking (a few days later than the second picking of pods) to the second picking (a few days later than the fourth picking of pods). The average level of ascorbic acid in leaves was much lower in the spring than in the fall (121.4 mg. vs. 158.6 mg. per 100 gm.) (table 4). The coefficients of variability for spring and fall were 10.55 percent and 13.36 percent, respectively. In the spring the most divergent replicate differed by 6.09 percent from the mean, while in the fall the most divergent replicate differed by 7.19 percent. The average content of the leaves for the average of spring and fall harvests varied from 123.6 mg. to 157.8 mg. per 100 gm.

TABLE 4.—*Average ascorbic acid content of 49 strains or varieties of snap beans in relation to weather during the 48 hours before picking, Charleston, S. C., 1941*

[196 samples at each picking date]

PODS

Crop and picking date	Ascorbic acid content per 100 gm. (fresh-weight basis)	Weather conditions		
		Sunshine	Average temperature	Rainfall
Spring crop:	*Milligrams*	*Hours*	*° F.*	*Inches*
May 27	24.41	26	75	0
June 2	24.61	12	80	0
June 8	21.96	25	77	0
June 14	22.60	17	78	0
Average (784 samples)	23.39			
Fall crop:				
Oct. 21	12.16	2	75	0.32
Oct. 28	21.47	16	70	0
Nov. 3	21.32	13	70	.27
Nov. 10	26.73	20	53	0
Average (784 samples)	20.42			
Significant difference M_{196}:				
5-percent level	.68			
1-percent level	.89			
Significant difference M_{784}:				
5-percent level	.39			
1-percent level	.45			

LEAVES

Crop and picking date	Ascorbic acid content per 100 gm. (fresh-weight basis)	Sunshine	Average temperature	Rainfall
Spring crop:				
June 5	128.64	21	64	0
June 17	114.22	15	49	0
Average (392 samples)	121.44			
Fall crop:				
Oct. 31	179.79	11	64	0
Nov. 12	137.38	15	49	0
Average (392 samples)	158.58			
Significant difference M_{196}:				
5-percent level	3.84			
1-percent level	5.05			
Significant difference M_{392}:				
5-percent level	2.72			
1-percent level	3.57			

The average amount of ascorbic acid in the spring crop of pods was 19.26 percent of that in the leaves. In the fall this value dropped to 12.88 percent. On the basis of absolute amounts the fall average of pods was 87.26 percent of the spring amount, but the spring average for leaves was only 76.58 percent of the fall average.

In both spring and fall there was a nonsignificant negative correlation of the ascorbic acid content of the first and second harvest of pods with that of the first harvest of leaves, −.034 and −.007. In the case of the third and fourth harvest of pods and the second harvest of leaves there were positive correlations of +.444**[5] and +.302*.

The manganese content of the soil was positively correlated with the ascorbic acid content of the spring crop of pods, +.173*, and negatively correlated with the content of the spring harvest of leaves, −.136. The manganese content of the soil varied from 55 to 329 p. p. m., with a mean content of 167 p. p. m. for the plots on which the spring crop was grown.

DISCUSSION

Preliminary investigations indicated that stage of maturity was not associated with large differences in the ascorbic acid content of pods. This finding confirmed that of Mack, Tapley, and King (*7*), who reported that there was not much difference between immature and overmature pods, and that the least ascorbic acid occurred in beans of the mature marketable stage. The differences between the three classes were much less than those found by Mack, Tapley, and King. Most of the analyses of pods reported in this investigation (table 3) were made on pods of marketable maturity.

Storage at 36° F. was much more effective in conserving ascorbic acid than storage at room temperature (70°). However, up to 48 hours there was not much difference in conservation of values between refrigeration and room temperature. After 48 hours there was a sharp drop in the ascorbic acid content of pods held at room temperatures, but under refrigeration the sharp drop did not come until after 72 hours. In a week at room temperature pods lost approximately half their original ascorbic acid content. Mack, Tapley, and King (*7*), using four varieties of snap beans, three sets of temperatures, and 6 days of storage, found markedly greater losses for both storage periods and temperatures than were found in these experiments. Humidity may have some bearing on this, since Mack, Tapley, and King stored at 60 to 70 percent relative humidity, and in the experiment reported here the relative humidity for the 36° storage groups was 98 to 100 percent. That humidity has a bearing on the retention of ascorbic acid is indicated by Harris, Wissmann, and Greenlie (*5*). They indicated that the rate of destruction of the vitamin in six vegetables, including snap beans, stored at an average relative humidity slightly less than 65 percent averaged about 64 percent greater than in corresponding samples stored at an average relative humidity of 93 percent.

Analyses of various parts of the bean plant indicated that roots do not store much ascorbic acid and that stems and pods have much less than leaves. These results agree quite well with those obtained

[5] Values marked ** are statistically significant by odds of 99 : 1 or more; values marked * are significant by odds of 19 : 1 or more, but less than 99 : 1.

by Reid (*10*) in a study of the distribution of ascorbic acid in cowpea plants.

The small correlations obtained between manganese content of the soil and ascorbic acid content of pods and of leaves indicated that in this experiment the manganese content was not a limiting factor although varying from 55 to 329 p. p. m. of soil. Hester (*6*) found a positive correlation between the manganese content of soil and the ascorbic acid content of tomatoes.

Varieties with large pods ranked highest in ascorbic acid content, but some of the smaller podded varieties also ranked high. Large-podded varieties used in this study also have relatively large seeds, and the pods tend to fill out more quickly than those of the small-podded strains. The varieties ranking highest in the Mack, Tapley, and King study (*7*) were those showing the same tendencies. The first four ranking strains all have large, market types of pods, while VBL 41, with the rank of 5 for a combination of both fall and spring, has a smaller, canning type of pod. The flat-podded types, Bountiful (No. 48) and VBL 46, ranked 1 and 2 and the oval-podded types, Stringless Black Valentine (No. 49) and VBL 28, ranked 3 and 4; while round types were not represented until 5, 6, and 7, with VBL 41, VBL 31, and VBL 17, respectively. That flatness is not closely associated with high ascorbic acid content was indicated by the fact that the flat strain VBL 14 had a rank of 30.

Leafiness is a characteristic that might be expected to influence the ascorbic acid content of pods, since in citrus fruits it has been found that those fruits which are more exposed to sunlight on the outer parts of a tree have a greater ascorbic acid content than the more shaded fruits from the inner parts of the tree (*3*, *4*). VBL 46 and Bountiful (No. 48) have a few large leaves and the pods are much exposed to sunlight, whereas VBL 19 and VBL 41 have an abundance of leaves which exclude a good deal more sunlight than the first two mentioned. The average (spring and fall) ascorbic acid content of the pods of VBL 46 and Bountiful was much greater than that of VBL 19 and VBL 41, but in the spring there was not much difference. In the early fall when there was less sunshine but hotter weather the ascorbic acid content of VBL 19 fell off sharply, but later in the fall when temperatures were lower and light intensity was further reduced, it approached that of Bountiful.

A study of the rankings of the pods of the 49 strains or varieties studied (table 3) indicates that on the average a strain which had a relatively high level of ascorbic acid in the spring likewise had a high level in the fall, and vice versa. Bountiful (No. 48), for instance, ranked 1 in the spring and 2 in the fall, while VBL 6 ranked 49 in both seasons. However, there were important exceptions to this general rule. VBL 19, which ranked 5 in the spring, ranked 32 in the fall, and VBL 5, which ranked 43 in the spring, ranked 14 in the fall. Possibly some of these interactions of ascorbic acid content with season may be part of the response of certain strains to length of day. Possibly, also, if ascorbic acid is needed in the metabolism of the bean plant, the leaves may utilize more and those of different varieties may utilize different quantities of it during periods of adverse weather. That ascorbic acid is needed in the metabolism of the plant is indicated by Reid (*11*). When the pods of VBL 19 ranked 5 in the spring, the leaves (table 3)

ranked 45; but, when the pods ranked 32 in the fall, the leaves ranked 7 in ascorbic acid content.

VBL 1 to 8, Brittle Wax (No. 9), and VBL 15 to 27, inclusive, are wax-podded strains; all others are green-podded. These 22 wax-podded strains had on an average much less ascorbic acid in the pods than the 27 green-podded strains, but that high content is not closely associated with the green-podded condition was indicated by the high rank attained by VBL 17 (a wax-podded type), which ranked 7 for spring and fall combined.

The two strains which had the highest average ascorbic acid content in the pods were strikingly different in the ascorbic acid content of the leaves. Bountiful (No. 48), which ranked 1 for content of pods, ranked 7 for leaves, while VBL 46, which was 2 for content of pods, ranked 42 for leaves (based on the averages for spring and fall combined). Considering all strains in the spring, there was a very small nonsignificant negative correlation (−.034) between first and second harvest of pods and first harvest of leaves. In the fall the correlation for these items was −.007. Between the third and fourth harvests of pods with second harvest of leaves the correlation was +.444** for spring and +.302* for fall.

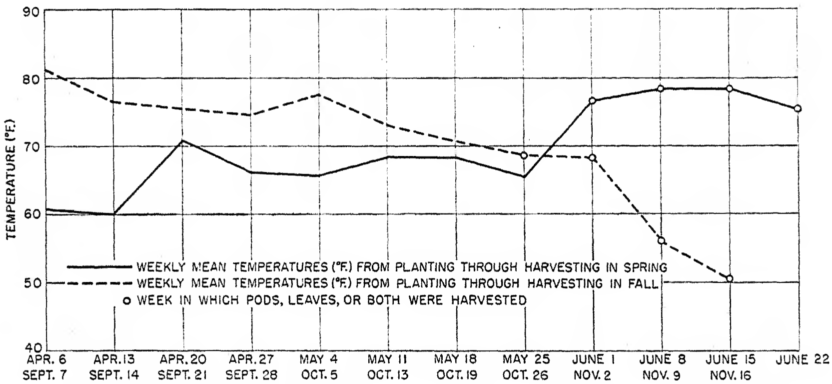

FIGURE 1.—Mean temperatures during the growing of two bean crops at Charleston, S. C.

Bountiful has light-green leaves and pods, Stringless Black Valentine has dark leaves and dark pods, and VBL 46 has medium-dark leaves and pods. For the total of all harvests the pods of these varieties ranked 1, 3, 2 and the leaves 7, 27, 42, respectively, in ascorbic acid content. This indicates that it is doubtful whether any significant relation exists between the intensity of green pigmentation of the strains and the amount of ascorbic acid in the pods.

The distribution of the temperatures for the spring and fall crops was strikingly different (fig. 1). The average temperatures based on weekly means were almost identical for the entire growing period, i. e., approximately 70° F. in both cases. Despite the great difference in weather conditions preceding the first pod harvest in the spring and that in the fall, both the lowest and the highest average ascorbic acid content were found in the fall crop, and (excluding the first fall pod harvest average) the spring and fall means were nearly identical (23.39

and 23.17, respectively). It appears that the ascorbic acid content of the pods was not much influenced by the weather during the period from planting to harvest. Mack, Tapley, and King (7) reported no significant seasonal effect over a period of 3 years. Considering the differences in soil and climatic conditions between Geneva, N. Y., and coastal South Carolina, some of the results are strikingly similar. For instance, Mack, Tapley, and King (7) found a value for U. S. No. 5 Refugee identical with that found here (19 mg. per 100 gm.); for Blue Lake they found a value of 11 mg., while that for Charleston was 12 mg.

A report of the sunshine, temperature, and rainfall during the 48-hour period just prior to each harvest (table 4) was obtained from the local station of the United States Weather Bureau, 8 miles away. It is noticeable that the October 21 harvest with its very low average was preceded by a 48-hour period during which there were only 2 hours of sunshine. Since carbohydrate substances are known to be low during periods of low light intensity, it might reasonably be expected that a low concentration of ascorbic acid would be found in the pods during a period of low light intensity extending over a period of 2 days. That temperature during this period was not an especially important factor is indicated by the fact that, although the temperature was lowest for the highest ranking harvest of pods, it was highest for the next highest ranking harvest.

Although the average for the pods was significantly lower for fall than for spring, the reverse was true for the leaves, and the lowest value obtained for the leaves in the fall was higher than the highest values obtained for the leaves in the spring. The second picking of leaves in both spring and fall averaged much below the first picking in each case, suggesting that the problem is one of translocation in the course of which the leaves tend to become somewhat exhausted of their ascorbic acid. The data in table 2 suggest that there is a translocation gradient from leaves to stems to pods to roots. The problem is somewhat complicated by the fact that in the spring there was a very dry windy period during May and June in which water losses from the leaves were so high that temporary wilting of the leaves occurred despite adequate applications of irrigation water. Although the fall crop was grown at a slightly higher average temperature, no wilting of leaves was observed at any time. There is also the additional complication that in the spring the days were becoming longer, while in the fall they were becoming shorter.

The coefficients of variability for ascorbic acid content of pods for spring and for fall were less than the corresponding coefficients of variability for yield of snap beans from the same plants and less than those usually found for snap bean and seed bean yields at the Charleston station. The soil on which the crops were grown was moderately uniform, and there was not much variation in ascorbic acid from one replicate to another. Most of the differences found could be accounted for by differences in variety and season. In no case did any picking or any combination of pickings of pods or of leaves fail to show significant varietal differences. The first picking of pods in the fall differed significantly from all other pickings and was the major factor in the significant seasonal difference observed. These results

contrast strikingly with those obtained by Currence (*2*) in experiments with tomatoes grown under field conditions in Minnesota. He found some interaction of variety and season, but in general the varieties that were high in ascorbic acid in the spring were also high in the fall, and vice versa. It is probable that the coefficients of variability would have been somewhat less if all the 46 VBL strains had been homozygous. Many of these strains were still segregating for various horticultural characteristics, and consequently any genetic variability for ascorbic acid content that may have occurred would be confounded with the random error component of the variance analysis.

SUMMARY AND CONCLUSIONS

In preliminary experiments it was found that stage of maturity—immature, marketable, and overmature—made only very slight differences in the ascorbic acid content of snap beans. Marketable pods were used in all the varietal experiments.

All pods and leaves used in the varietal experiments were analyzed either immediately or within 48 hours after storage at 36° F. Storage at 36° prevented loss of ascorbic acid much better than storage at room temperature, but there was not much difference in the two storage treatments until after 48 hours. The leaves had the highest ascorbic acid content, followed in order by the stems, pods, and roots, suggesting a translocation gradient.

In an experiment carried out in the fall of 1941 with several strains and varieties of beans, it was found that in general the higher quality varieties, such as Blue Lake, U. S. No. 5 Refugee, and Kentucky Wonder, had much less ascorbic acid than more fibrous beans, such as the field bean Idaho No. 1 Mosaic Resistant Great Northern.

In the major experiment reported, 46 hybrid strains and 3 commercial varieties were arranged in a lattice square in 4 replicates, and ascorbic acid determinations were made from each of 4 pickings of pods from a spring planting and the same number from a similar fall planting. Determinations were made on leaves corresponding as closely as possible in each case to second and fourth pickings of pods.

There were significant varietal differences for each picking and for the average of all pickings. The strains tested varied from 19.1 mg. per 100 gm. to 28.7 in the spring, from 16.2 to 26.4 in the fall, and from 17.7 to 27.5 for fall and spring combined. The variety with the highest ascorbic acid content was Bountiful (No. 48), although there was no significant difference between it and hybrid VBL 46, which resembles it. All other strains contained significantly less ascorbic acid than Bountiful on the basis of the complete set of data for pods. Although high-quality strains contained significantly less ascorbic acid than Bountiful, some were sufficiently close to justify the belief that it is possible to combine high quality with a very high ascorbic acid content.

In general, if a strain ranked high or low in ascorbic acid in the spring, it had a similar rank in the fall, although there were enough exceptions to indicate that some strains would rank much higher under one set of conditions than under another.

There were very significant differences between strains in respect to the ascorbic acid content of their leaves. The leaves were always

much higher in ascorbic acid than the pods. The lowest average content of leaves in the fall was higher than the highest content in the spring.

Consideration was given to ascorbic acid content in relation to size of pod, leafiness of plant, waxiness or greenness of pods, intensity of greenness in leaves and pods, and weather conditions at harvesting time and to correlations between ascorbic acid content of pods and that of leaves.

A small, significant, positive correlation was found between manganese in the soil and the ascorbic acid content of the combined harvests of pods for spring, and a small negative, nonsignificant correlation between manganese in the soil and the ascorbic acid content of the combined harvests of leaves for spring.

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EFFECT OF PHOTOPERIOD ON RICE VARIETIES GROWN IN THE FIELD[1]

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INTRODUCTION

Hundreds of varieties of rice (*Oryza sativa* L.) have been introduced into the United States from the principal rice-producing countries of the world for possible use in rice improvement. When varieties introduced from the Tropics are grown in the Southern States, they often fail to head or else head so late that they do not set seed or mature before the plants are killed by cold. This behavior in the United States apparently is due to the longer days, which delay heading, and to the cooler weather, which retards maturity, as compared with conditions in the Tropics.

Many tropical varieties, when grown under long natural daylight periods in temperate regions, make a rather vigorous vegetative growth and have a tendency to continue growth until the short days of late autumn. They may then head slowly and unevenly, set a few seed on comparatively long panicles, but fail to mature normally. On the other hand, some late-maturing varieties from temperate regions, when grown in the Tropics under relatively short-day conditions, produce rather small plants with few tillers, bearing short panicles that set seed and mature much earlier than in temperate climates.

Fortunately, certain varieties from the Tropics head early enough to mature seed in the South, and these or selections from them have proved to be well adapted for growing there. Rice breeders are fortunate in that among the rice varieties grown in various parts of the world there may be found marked differences in physiological response to light and temperature conditions.

The known photoperiodic response of rice varieties can be used to advantage by rice breeders in controlling the time of heading of varieties to be used in hybridization. Also, information as to varietal reaction to early and late seeding can be obtained by tests of photoperiodic response. The present paper reports the effect on time of heading and plant growth of different day lengths obtained by covering field-grown plants to exclude light for various periods.

REVIEW OF LITERATURE

The effect of the relative length of day and night upon the time of flowering of plants, reported by Garner and Allard (*4, 5, 6*),[2] has been studied by numerous investigators working with rice. In pot experiments, in which a few plants per treatment were used, Eguchi (*2*),

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[2] Italic numbers in parentheses refer to Literature Cited, p. 340.

Fuke (*3*), Kondo et al. (*9, 10*), Miyashiro (*11*), Noguchi (*12, 13*), Tabata et al. (*15*), and Yoshii (*16*) all report a reduction in the number of days required for rice to reach the heading stage when the length of day was shortened. In date-of-seeding experiments in pots and under field conditions, Adair (*1*), Jenkins (*7*), and Scripchinsky (*14*) reported a reduction in the time required for rice to mature as date of seeding was delayed.

Tabata et al. (*15*) limited Japanese rice varieties to 8 hours of sunlight per day as early as 54 days after seeding. The earliest treatments reduced the time from seeding to heading as much as 22 days, whereas the treatments begun after the plants were well advanced had no effect on time of heading. The short-day treatments reduced the yield of grain.

Miyashiro (*11*) found that a 10-hour daylight period, beginning 84 to 86 days after seeding, hastened heading by about 1 week. He also reported that light interception during the morning delayed heading 8 to 9 days, whereas afternoon light interception had no effect on time of heading.

Yoshii (*16*) used a 9-hour light period, beginning at an early stage of plant development, and reduced the time required from seeding to heading of late varieties by about 2 weeks. He also reported that daily light periods of 5 and 9 hours, especially with late-maturing varieties, increased the number of tillers per plant and slightly reduced the length of panicles, yield of grain, and plant height.

Fuke (*3*) concluded that, as a rule, short-day treatments are most effective when used on plants that have seven to nine leaves and are beginning to tiller freely. Treatments starting 30 days prior to natural heading are slightly less effective, and treatments beginning before the plants have seven to nine leaves, or 16 days prior to natural heading, are relatively ineffective.

Kondo et al. (*10*) found that short-day treatments increased the number of tillers and had no effect on length of panicles but caused a reduction in grain yield and plant height. They also reported that rice grown under 8 hours of light per day, divided into two or three periods in which the over-all time from beginning to end of photoperiod was 12 hours, headed at essentially the same time as when grown under a continuous 8-hour light period. When grown with 12 hours of light, divided into two periods in which the over-all time from beginning to end of photoperiod was 16 hours, the plants reacted as to a long day and did not head. When grown with 12 hours of continuous light, the plants reacted as to a short day, and heading was accelerated. They also found that the time of heading of an extremely early variety from Hokkaido, Japan, grown under a short-day treatment, was unaffected, whereas the time of heading of an extremely late Formosan variety was greatly accelerated by the short day.

In other studies, Kondo et al. (*9*) limited the daily light periods from transplanting to heading to 8 or 12 hours. The treatments reduced the time to heading by as much as 1 to 2 months. They also reported two periods of heading for seedlings limited to 8 or 12 hours of daylight during the entire seedbed period followed by normal daylight. The first period was much earlier and the second later than normal.

Eguchi (*2*) placed early- and late-maturing rice varieties in different groups based on their reaction to short-day treatments. In late-

maturing varieties, the short-day treatment accelerated the differentiation of flower buds but, when continued, had no effect upon subsequent development of the differentiated buds. In the case of early-maturing varieties, both time of differentiation and further development of flower buds were found to be independent of day length.

Jenkins (*7*), in commenting on date-of-seeding experiments with rice, stated that—

> Some varieties tend to have a comparatively fixed growing period, while in others it appears that heading will not take place until a certain time in the fall after which their growing periods shorten materially.

Date-of-seeding experiments reported by Adair (*1*) show that the rice varieties referred to as "sensitive" decreased markedly in plant height and in time required to reach the heading stage as the seeding was delayed. Varieties referred to as "indifferent" in most cases showed no consistent differences in plant height and smaller and less consistent differences in time required to reach the heading stage as the date of seeding was delayed.

MATERIAL AND METHODS

In 1936, 10 varieties of rice, growing in the field at substation No. 4 of the Texas Agricultural Experiment Station at Beaumont, Tex., were subjected to controlled photoperiods during different stages of growth. Jones (*8*) has described 6 of the varieties grown in this study. Of the remaining 4, Kameji is a midseason, short-grain Japanese variety and C. I.[3] 6993 is a very early, short-grain Japanese variety. C. I. 81C and C. I. 4700, medium-grain varieties that tiller profusely, were introduced from the Philippine Islands. The first-named variety matures early, while the latter matures very late.

A single row 203 feet long of each variety was sown on April 13. The rows were spaced 8 inches apart and the seeds were spaced 4 inches apart within the rows. The first seedlings emerged on April 29, and all seedlings had emerged by May 6. The area was divided into 58 plots, and each plot included a 3½-foot-row section of each variety. The treated plots were distributed in a systematic fashion along with 4 untreated or normal-day plots. The treated plots were duplicated.

The length of day was controlled by covering the plants for definite periods with frames covered with mumshade cloth.[4] These frames were 3½ feet wide, 8 feet long, and 2½ feet high. Medium-weight unbleached muslin was placed outside the mumshade cloth to reflect heat and thus assist in maintaining a nearly normal temperature under the cover.

The plants were covered daily for 10-day periods beginning June 1, 11, and 21, and July 1, that is, 49, 59, 69, and 79 days after seeding; for 20-day periods beginning June 1, 11, and 21; and for 30-day periods beginning June 1 and 11. On June 1 the plants were in the fourth-leaf stage and, with but few exceptions, had not started to tiller. Three length-of-day treatments, on adjacent plots, were obtained by covering the plants for the following periods: (1) From 6 p. m. to 8 a. m.; (2) from 8 a. m. to 1 p. m.; and (3) from 1 to 6 p. m.

The length and distribution of light and dark periods for the treatments and the natural days are shown in figure 1. Covering periods

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.
[4] A black cloth for preventing light infiltration.

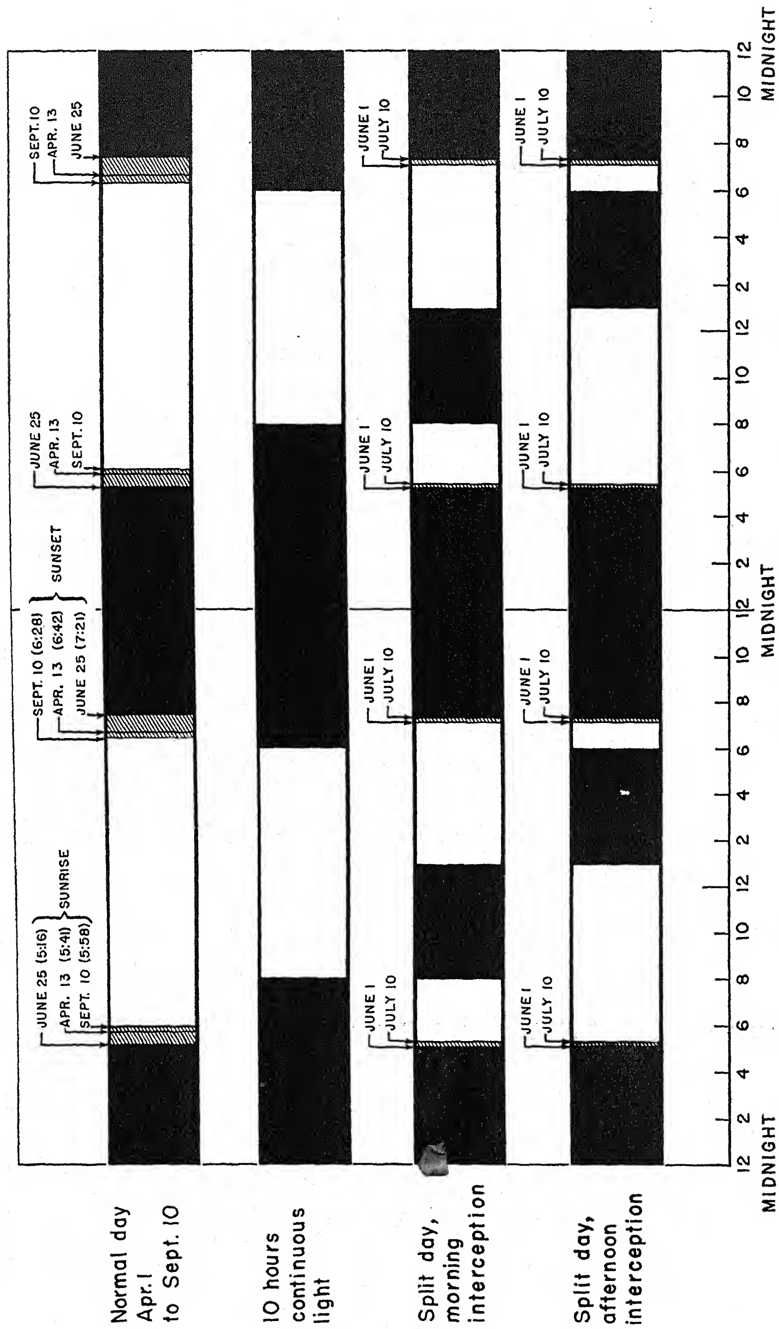

FIGURE 1.—Length and distribution of various light periods studied and length of the normal day on April 13, June 25, and September 10.

2 and 3 are referred to as the split-day treatment, because the plants received light from daybreak until covered and from the end of the covering period until darkness.

The number of hours between sunrise and sunset during the growing season ranged from 13 hours 1 minute, on April 13, to 14 hours 5 minutes, on June 25. By September 10 the normal day had been reduced to 12 hours 30 minutes. On June 1, when the covering treatments were started, and on July 10, when they ended, there was 13 hours 57 minutes between sunrise and sunset.

Data were obtained on an average of 14.6 plants of each variety for each covering period and 29.9 plants of each variety for the control (plants of normal-daylight period), making a grand total of 4,227 plants in all treatments. The various characters studied for each plant were: (1) Date of first panicle emergence from the boot; (2) number of tillers and panicles; (3) length of panicle on tallest tiller and height of tallest tiller; (4) average grain and straw weights. The average grain and straw weights are based on the total weights of grain and straw per row divided by the number of plants. Student's test for unique samples was used to determine whether the differences between plots covered from 8 a. m. to 1 p. m. and those covered from 1 to 6 p. m. were significant.

EXPERIMENTAL RESULTS

TIME OF HEADING

The effect on the number of days required from seeding to first panicle emergence of 10-hour photoperiods for 10, 20, and 30 days, beginning at different stages of growth, is shown in table 1. The varieties were placed in two groups, based on their reaction to the short-day treatments. In group 1, the "sensitive" varieties, a marked reduction in the number of days required from seeding to heading

TABLE 1.—*Effect on time of panicle emergence in 10 rice varieties of 10-hour photoperiods for 10, 20, and 30 days at different stages of growth*

Group and variety	Average plants per treatment	Average period to panicle emergence of control	Average period from seeding to first panicle emergence when covered daily for—								
			10 days beginning—				20 days beginning—			30 days beginning—	
			June 1	June 11	June 21	July 1	June 1	June 11	June 21	June 1	June 11
Sensitive varieties:	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
C. I. 81C	13.9	107.1	88.3	90.6	97.3	103.4	80.3	86.6	95.3	80.6	87.6
Caloro	12.9	114.2	86.6	91.2	98.2	107.4	83.1	88.0	96.5	80.7	88.0
Kameji	15.1	121.2	81.8	89.0	96.8	108.9	78.0	85.9	94.4	78.4	85.1
Improved Blue Rose	17.2	131.0	90.7	92.4	101.0	112.4	80.5	87.7	97.1	82.4	87.1
C. I. 4700	17.3	146.0	89.9	95.4	106.7	119.2	79.6	87.5	97.3	77.3	87.3
Average	15.3	123.9	87.5	91.7	100.0	110.3	80.3	87.1	96.1	79.9	87.0
Less sensitive varieties:											
C. I. 6993	5.6	85.5	78.8	83.0	85.8	86.4	77.2	81.4	86.2	80.0	84.0
Shoemed	15.4	115.7	113.8	103.8	103.0	108.7	96.4	94.3	100.0	93.5	93.2
Nira	16.0	124.6	126.6	124.4	118.2	118.1	125.3	114.8	110.8	117.7	110.6
Fortuna	15.9	124.8	125.2	125.0	119.5	117.4	125.4	117.3	111.7	123.5	114.3
Rexoro	15.0	145.8	147.6	145.3	129.8	125.6	147.6	120.5	112.5	138.9	109.5
Average	13.6	119.3	118.4	116.3	111.3	111.2	114.4	105.7	104.2	110.7	102.3

occurred for the earliest 10-, 20-, and 30-day covering periods, followed by a more gradual and less marked reduction as the date of covering was delayed. While there was a progressive increase in the number of days required to head for the successively later covering periods, all varieties treated headed earlier than the controls. The smallest reduction was 3.7 days for C. I. 81C, covered daily for 10 days beginning July 1, and the largest reduction was 68.7 days for C. I. 4700, covered 30 days starting June 1. The 10-, 20-, and 30-day covering periods had the same general effect on date of heading, but the longer covering periods showed slightly more effect.

All sensitive varieties, except a few plants and C. I. 81C, when covered for 10 days starting June 1, headed during two periods. The variety C. I. 4700 also reacted in this manner to a limited extent for the 10-day covering period beginning June 11. The short covering periods, at this stage of seedling development, caused the tillers on plants of these varieties to head early, whereas the tillers produced during and after the covering period were not affected by the short-day treatment and headed at essentially the same time as the control plants. Kondo et al. (*10*) also reported two periods of heading; the first was much earlier and the second later than normal.

The tabular data reported in this part of the paper on the time of heading, number of panicles, length of panicles, plant height, and the weight of grain and straw for the sensitive varieties, except C. I. 81C, for these covering periods (June 1 and June 11 for 10 days), are based on information recorded for the tillers that headed early. No information on the plant characters studied was recorded for the tillers that headed late for these particular varieties and treatments. However, it was observed that the late tillers were about normal in height and produced panicles that appeared to be of essentially the same length as those of plants of the normal-day length.

In group 2, the less sensitive varieties, the earliest covering periods for 10, 20, and 30 days showed in general a slight reduction in the number of days required from seeding to heading, followed by a gradual but more marked reduction as the date of covering was delayed. The variety C. I. 6993 did not behave in this manner but showed a maximum reduction in the time required to head for the earliest covering periods. This very early variety was placed in the less sensitive group because its response to the short-day treatments was not very marked. The other exception to the usual reaction of the less sensitive varieties was Shoemed when covered 10 and 20 days beginning July 1 and June 21 respectively. The reactions of the less sensitive varieties ranged from an increase of 2 days for Nira when covered 10 days beginning June 1, to a reduction of 36.3 days for Rexoro when covered 30 days beginning June 11.

The average effect of the short-day treatments on the number of days to heading, number of tillers and panicles, length of panicles, plant height, and the grain and straw weights per plant are shown in figure 2.

NUMBER OF TILLERS

The average number of tillers and panicles for the various covering periods is shown in table 2. The 10-day covering period beginning June 1 resulted in a marked increase in the number of tillers for the sensitive varieties Caloro, Kameji, Improved Blue Rose, and C. I.

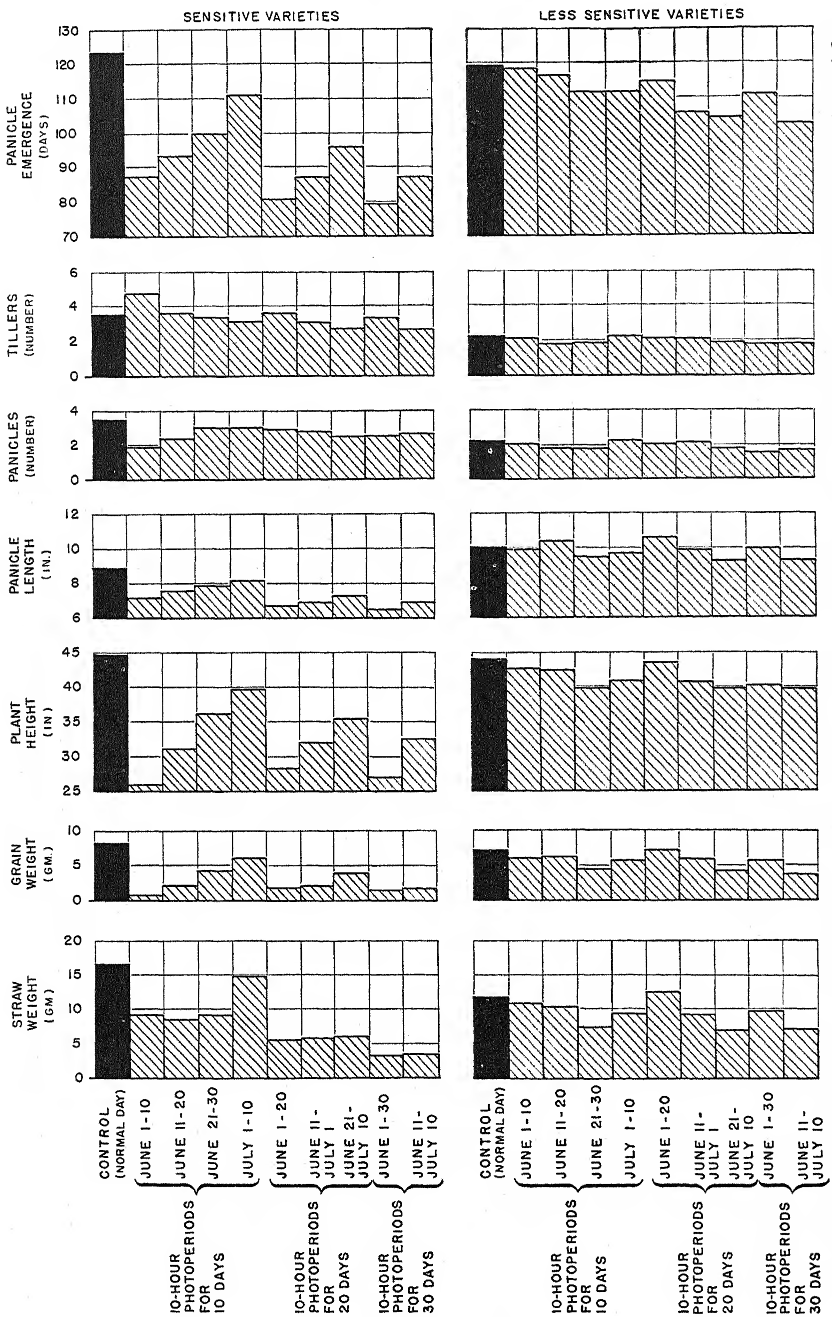

FIGURE 2.—Average effect of the short-day treatments on the number of days to heading, number of tillers and panicles, length of panicles, plant height, and grain and straw weights per plant.

4700, which headed during two periods, but not for C. I. 81C. The other short-day treatments, for both the sensitive and the less sensitive varieties, resulted in only slight increases or decreases in the number of tillers for the earliest covering periods, while for the later covering periods they brought about a gradual reduction in number. The greatest reduction was observed in plants covered 30 days (fig. 2). There was more variation in the number of tillers for the sensitive than for the less sensitive varieties.

TABLE 2.—*Effect on number of tillers and panicles per plant in 10 rice varieties of a 10-hour photoperiod for 10, 20, and 30 days at different stages of growth*

Group and variety	Average plants per treatment	Average tillers and panicles of control	Average tillers and panicles per plant when covered daily for—								
			10 days beginning—				20 days beginning—			30 days beginning—	
			June 1	June 11	June 21	July 1	June 1	June 11	June 21	June 1	June 11
TILLERS											
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Sensitive varieties:											
C. I. 81C	12.8	4.61	4.50	3.25	3.87	2.35	3.85	3.62	2.77	3.58	2.83
Caloro	12.4	2.85	3.60	2.81	2.92	2.86	3.57	2.14	2.27	2.92	1.50
Kameji	12.3	2.78	4.00	2.75	2.26	2.67	3.18	2.29	2.25	2.83	2.83
Improved Blue Rose	13.2	3.17	4.00	2.84	2.64	2.69	3.38	3.36	2.59	3.33	2.00
C. I. 4700	16.0	4.50	7.85	6.47	5.05	5.00	4.14	3.76	3.50	4.11	4.18
Average	13.3	3.58	4.79	3.62	3.35	3.11	3.62	3.03	2.68	3.35	2.67
Less sensitive varieties:											
C. I. 6993	5.6	2.55	2.67	2.17	2.17	2.60	2.40	2.43	2.20	2.20	1.80
Shoemed	14.9	2.97	2.38	2.00	1.79	2.80	2.92	2.67	2.44	1.93	1.90
Nira	16.0	1.97	1.82	1.55	1.94	2.06	2.00	1.72	1.52	1.56	1.74
Fortuna	15.9	1.91	1.59	1.88	1.76	2.21	1.82	1.93	2.08	1.65	1.81
Rexoro	15.0	1.90	2.07	1.75	1.80	1.67	1.82	2.00	1.53	1.92	2.00
Average	13.5	2.26	2.11	1.87	1.89	2.27	2.19	2.15	1.95	1.85	1.85
PANICLES											
Sensitive varieties:											
C. I. 81C	12.8	4.43	3.57	2.75	3.80	2.35	3.85	3.62	2.69	3.17	2.83
Caloro	12.4	2.73	2.20	2.25	2.85	2.79	3.14	2.00	2.27	2.83	1.50
Kameji	12.3	2.78	1.62	1.88	2.21	2.60	2.91	2.00	2.17	2.17	2.83
Improved Blue Rose	13.2	3.11	1.08	2.05	2.41	2.63	1.88	3.00	2.29	1.67	2.00
C. I. 4700	16.0	4.50	1.08	3.18	3.95	4.76	3.07	3.47	3.17	2.94	4.18
Average	13.3	3.51	1.91	2.42	3.04	3.03	2.97	2.82	2.52	2.56	2.67
Less sensitive varieties:											
C. I. 6993	5.6	2.55	2.67	2.17	2.17	2.60	2.40	2.43	2.20	1.80	1.60
Shoemed	14.9	2.84	2.38	2.00	1.74	2.67	2.17	2.61	2.13	1.36	1.90
Nira	16.0	1.97	1.65	1.55	1.88	2.00	2.00	1.72	1.52	1.38	1.74
Fortuna	15.9	1.91	1.47	1.88	1.59	2.14	1.76	1.93	2.08	1.65	1.63
Rexoro	15.0	1.90	2.07	1.75	1.67	1.67	1.71	1.92	1.53	1.75	1.92
Average	13.5	2.23	2.05	1.87	1.81	2.22	2.01	2.12	1.89	1.59	1.76

NUMBER OF PANICLES

On the whole, short-day treatments reduced the average number of panicles per plant for both the sensitive and less sensitive varieties (table 2). Neither group of varieties showed consistent increases or decreases in the number of panicles for the different covering periods, except for the sensitive varieties that headed during two periods as a

result of early covering (fig. 2). These varieties for the 10-day covering period beginning June 1, and June 11 for C. I. 4700, if both early and late tillers produced panicles, would probably show an increased number as compared with the control plants.

LENGTH OF PANICLE

The average panicle length for the various covering periods is shown in table 3. The average panicle length of the sensitive varieties was greatly reduced when the plants were covered 10, 20, or 30 days starting June 1; later covering periods showed less reduction. The less sensitive varieties showed little variation in panicle length for the different covering periods. However, the panicles in the later covering periods were slightly shorter than those of the controls, while in the early covering periods they showed no reduction (fig. 2).

TABLE 3.—*Effect on panicle length and plant height in 10 rice varieties of 10-hour photoperiods for 10, 20, and 30 days at different stages of growth*

PANICLE LENGTH

Group and variety	Average plants per treatment	Average panicle length and plant height of control	Average length of panicle on tallest tillers and height of each plant when covered daily for—								
			10 days beginning—				20 days beginning—			30 days beginning—	
			June 1	June 11	June 21	July 1	June 1	June 11	June 21	June 1	June 11
Sensitive varieties:	*Number*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
C. I. 81C	12.8	8.9	8.1	7.9	8.0	8.2	7.2	7.1	7.7	7.3	7.3
Caloro	12.4	8.4	7.5	7.4	7.9	7.7	6.9	6.6	7.0	6.7	6.4
Kameji	12.3	7.9	6.1	6.3	6.9	7.0	5.5	5.9	6.4	5.6	6.3
Improved Blue Rose	13.2	9.1	7.2	7.7	7.8	8.4	6.9	7.4	7.3	5.8	7.6
C. I. 4700	16.0	10.3	7.0	8.6	8.9	9.8	7.2	7.3	8.0	7.1	6.8
Average	13.3	8.9	7.2	7.6	7.9	8.2	6.7	6.9	7.3	6.5	6.9
Less sensitive varieties:											
C. I. 6993	5.6	7.1	6.4	6.8	6.7	6.6	6.3	6.8	6.4	5.7	6.6
Shoemed	14.9	10.5	10.0	11.6	10.4	10.3	12.0	10.1	9.7	10.4	9.4
Nira	16.0	11.6	11.7	12.0	10.8	10.9	11.9	11.1	10.5	11.7	10.9
Fortuna	15.9	10.5	10.6	10.5	9.6	10.4	11.5	10.7	10.3	11.2	10.3
Rexoro	15.0	10.8	10.9	11.0	9.8	10.5	11.3	10.7	9.7	10.8	9.4
Average	13.5	10.1	9.9	10.4	9.5	9.7	10.6	9.9	9.3	10.0	9.3

PLANT HEIGHT

Group and variety	Average plants per treatment	Average panicle length and plant height of control	10 days June 1	10 days June 11	10 days June 21	10 days July 1	20 days June 1	20 days June 11	20 days June 21	30 days June 1	30 days June 11
Sensitive varieties:											
C. I. 81C	12.8	42.9	34.1	36.9	39.5	41.7	32.9	34.5	38.5	31.1	33.5
Caloro	12.4	39.4	25.5	30.6	35.2	38.9	27.0	28.9	32.4	27.0	29.8
Kameji	12.3	42.1	20.5	26.1	34.4	35.5	22.4	27.4	32.3	23.1	31.3
Improved Blue Rose	13.2	46.1	23.8	29.3	34.2	37.6	28.5	33.8	34.4	24.8	34.0
C. I. 4700	16.0	54.0	25.0	33.1	37.3	44.8	31.3	35.5	39.1	29.7	33.7
Average	13.3	44.9	25.8	31.2	36.1	39.7	28.4	32.0	35.3	27.1	32.5
Less sensitive varieties:											
C. I. 6993	5.6	33.5	28.0	32.8	31.7	33.2	28.2	32.1	31.8	24.8	31.6
Shoemed	14.9	43.0	41.2	40.9	41.6	43.9	42.3	43.3	42.6	41.4	42.0
Nira	16.0	50.9	50.7	50.0	45.6	46.3	51.8	46.2	45.0	45.6	45.8
Fortuna	15.9	46.3	46.5	44.5	42.0	44.4	47.6	45.1	43.5	46.6	42.1
Rexoro	15.0	46.8	47.0	44.8	38.4	36.2	47.9	37.6	36.8	42.2	37.6
Average	13.5	44.1	42.7	42.6	39.9	40.8	43.6	40.9	39.9	40.1	39.8

PLANT HEIGHT

Average plant height, based on the tallest tiller of each plant, showed a marked reduction for the sensitive varieties at the earliest covering periods (table 3). As the date of covering was delayed, there was a gradual increase in plant height, but the latest covering periods still showed an appreciable reduction in height as compared with plants of the normal-day or control plot. The less sensitive varieties showed only slight reductions in plant height at the earliest covering periods, followed by greater reductions as the date of covering was delayed (fig. 2).

In both the sensitive and less sensitive groups, the 10-, 20-, and 30-day covering periods showed essentially the same effect on plant height, except that the effects of the short-day treatments were slightly more intensified for the longer covering periods.

GRAIN AND STRAW WEIGHTS

The average grain and straw weights were determined for the sensitive varieties Caloro, Improved Blue Rose, and C. I. 4700, and for the less sensitive varieties Shoemed, Nira, Fortuna, and Rexoro (table 4). As a group the sensitive varieties showed the greatest re-

TABLE 4.—*Effect on average grain and straw weights in 7 rice varieties of 10-hour photoperiods for 10, 20, and 30 days at different stages of growth*

Group and variety	Average plants per treatment	Average grain and straw weight of control	Average grain and straw weight per plant when covered daily for— 10 days beginning— June 1	June 11	June 21	July 1	20 days beginning— June 1	June 11	June 21	30 days beginning— June 1	June 11
GRAIN											
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Sensitive varieties:											
Caloro	12.4	5.7	1.3	2.3	4.7	6.0	2.7	1.6	3.6	1.3	1.3
Improved Blue Rose	13.2	7.5	.4	1.8	4.0	4.4	1.4	1.9	2.8	.5	2.2
C. I. 4700	16.0	12.0	.4	2.1	4.1	7.6	1.7	2.6	5.3	2.3	1.7
Average	13.9	8.4	.7	2.1	4.3	6.0	1.9	2.0	3.9	1.4	1.7
Less sensitive varieties:											
Shoemed	14.9	7.3	3.4	4.4	3.2	6.0	5.3	4.9	3.9	2.9	1.9
Nira	16.0	7.5	6.6	7.1	5.6	5.9	7.9	5.6	3.6	5.9	4.1
Fortuna	15.9	7.2	5.8	6.5	5.1	6.1	8.4	7.5	6.0	7.2	5.1
Rexoro	15.0	6.5	8.3	6.8	4.1	4.9	7.4	5.2	3.1	6.3	4.2
Average	15.5	7.1	6.0	6.2	4.5	5.7	7.3	5.8	4.2	5.6	3.8
STRAW											
Sensitive varieties:											
Caloro	12.9	8.0	5.7	5.3	5.7	8.7	3.3	3.1	3.9	2.1	2.0
Improved Blue Rose	17.2	15.5	8.9	7.5	7.4	11.2	5.8	6.0	6.4	4.1	3.7
C. I. 4700	17.3	26.4	13.1	12.6	14.5	24.4	7.4	7.8	7.8	4.1	4.9
Average	15.8	16.6	9.2	8.5	9.2	14.8	5.5	5.6	6.0	3.4	3.5
Less sensitive varieties:											
Shoemed	15.4	11.8	7.1	6.3	5.5	11.0	8.8	7.2	6.4	5.9	5.9
Nira	16.0	11.2	10.4	10.6	9.4	10.6	13.6	10.6	7.5	10.1	8.4
Fortuna	15.9	8.8	7.4	9.3	6.8	8.5	9.9	10.7	7.9	9.2	7.9
Rexoro	15.0	15.6	18.3	14.8	8.3	7.9	17.2	8.8	6.1	14.4	6 6
Average	15.6	11.9	10.8	10.3	7.5	9.5	12.4	9.3	7.0	9.9	7.2

duction in grain and straw weights at the earliest covering periods, except from the 10-day treatment beginning June 1 for straw weights. As in the case of the other characters studied, the effect was less the later the covering periods were begun. The less sensitive varieties showed only slight reductions in grain and straw weights, as compared with the sensitive varieties, at the earliest covering periods. There was a gradual intensification of the effect as the date of covering was delayed (fig. 2).

SPLIT-DAY TREATMENTS

As shown in table 5, plants subjected to morning and afternoon covering usually headed later than controls. The early-maturing varieties C. I. 6993 and C. I. 81C showed the greatest effect from the split-day treatments and, when covered daily from 8 a. m. to 1 p. m. or from 1 to 6 p. m. for 30 days beginning June 1, headed 19.2 and 12.1 days and 18.5 and 12.0 days later, respectively, than plants of the normal-day treatment. The delay in time of heading was not so marked for the midseason and late varieties Kameji, Nira, Fortuna, and Improved Blue Rose, ranging from 1.3 days earlier to 4.5 days later than the control plants. Rexoro and C. I. 4700, very late-maturing varieties, were only slightly affected by these treatments, ranging from 0.9 days earlier to 1.9 days later in heading than the control plants. The greatest delays in time of heading were for the 30-day covering period.

A rather consistent difference in varietal reaction to morning and afternoon interception of light was evident for the 10-, 20-, and 30-day covering periods ended on July 10. The less sensitive varieties Shoemed, Nira, and Fortuna, when covered from 8 a. m. to 1 p. m. for these periods, headed 1.7 to 11.0 days later than adjacent plants covered from 1 to 6 p. m. Rexoro behaved in the same manner, except that the differences were smaller. The sensitive varieties Caloro, Kameji, and Improved Blue Rose, of similar maturity as Shoemed, Nira, and Fortuna, when covered from 8 a. m. to 1 p. m. for 10, 20, and 30 days, ending July 10, headed 0.9 to 2.4 days earlier than adjacent plants covered from 1 to 6 p. m.; but no consistent differences in time of heading between morning and afternoon treatments appeared for C. I. 81C, C. I. 6993, and C. I. 4700.

The split-day plots, when covered on June 30 or later—including 12 treatments of each day length with a total for each variety of 11 to 38 control plants, 27 to 112 plants covered in the morning, and 28 to 110 plants covered in the afternoon—were compared to determine whether the differences between morning and afternoon light interception were significant statistically. The averages for the factors studied, based on the 12 treatments of each day length, are shown in table 6. While the differences recorded were small, they appear to be significant for some varieties. The sensitive varieties Caloro, Kameji, and Improved Blue Rose headed slightly earlier when covered in the morning than when covered in the afternoon, and the differences appear to be highly significant for each variety. All 5 of the less sensitive varieties and sensitive varieties C. I. 81C and C. I. 4700 headed slightly later when covered in the morning than when covered in the afternoon. The differences for Shoemed, Nira, Fortuna, and Rexoro were highly significant and that for C. I. 81C was significant.

TABLE 5.—*Comparative effect on time of panicle emergence in 10 rice varieties of split-day treatments from 8 a. m. to 1 p. m. and 1 to 6 p. m. for 10, 20, and 30 days at different stages of growth*

Treatment: Date ended	Treatment: Duration	Treatment: Clock time	Average plants per variety	Sensitive varieties: C. I. 81C	Sensitive varieties: Caloro	Sensitive varieties: Kameji	Sensitive varieties: Improved Blue Rose	Sensitive varieties: C. I. 4700	Sensitive varieties: Average	Less sensitive varieties: C. I. 6993	Less sensitive varieties: Shoemed	Less sensitive varieties: Nira	Less sensitive varieties: Fortuna	Less sensitive varieties: Rexoro	Less sensitive varieties: Average
	Days		*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Control			29.9	107.1	114.2	121.2	131.0	146.0	123.9	85.5	115.7	124.6	124.8	145.8	119.3
June 10	10	8 a. m.–1 p. m.	14.0	110.6	115.4	123.1	131.9	146.2	125.4	91.8	116.6	126.0	125.2	146.3	121.2
	10	1–6 p. m.	13.6	109.5	113.8	123.3	131.8	146.5	125.0	90.7	115.3	128.1	125.0	145.3	120.9
June 20	10	8 a. m.–1 p. m.	15.0	112.4	114.6	122.2	131.7	145.6	125.3	92.5	117.0	124.3	124.9	146.1	121.0
	10	1–6 p. m.	14.2	110.2	115.7	122.2	131.8	146.0	125.2	89.6	116.7	124.6	124.3	145.6	120.2
June 30	10	8 a. m.–1 p. m.	15.1	111.8	118.3	122.1	131.4	146.0	125.9	88.5	120.0	124.4	125.3	145.8	120.8
	10	1–6 p. m.	16.8	110.1	118.4	122.4	131.2	145.1	125.4	87.6	115.3	124.6	124.6	144.9	119.4
July 10	10	8 a. m.–1 p. m.	15.4	108.1	118.2	121.8	129.7	146.3	124.8	86.3	117.0	127.6	125.6	147.1	120.7
	10	1–6 p. m.	14.5	108.2	120.0	123.8	131.8	146.5	126.1	85.0	114.4	123.4	123.5	145.7	118.4
June 20	20	8 a. m.–1 p. m.	14.7	113.7	117.1	123.8	132.7	145.4	126.5	99.0	118.2	126.5	125.8	146.3	123.2
	20	1–6 p. m.	13.9	115.3	116.6	123.7	132.6	145.6	126.8	98.6	120.1	125.5	126.6	146.4	123.4
June 30	20	8 a. m.–1 p. m.	14.3	113.6	117.6	122.2	132.4	145.9	126.3	89.0	121.2	126.3	125.3	145.7	121.5
	20	1–6 p. m.	15.1	113.1	118.4	122.5	132.0	145.8	126.4	89.6	117.3	124.6	124.8	145.1	120.3
July 10	20	8 a. m.–1 p. m.	14.5	113.6	121.7	122.9	131.6	146.9	127.3	87.4	125.2	128.9	127.9	147.0	123.3
	20	1–6 p. m.	13.9	111.6	122.7	125.3	132.7	147.0	127.9	89.8	114.2	124.2	124.4	145.9	119.7
June 30	30	8 a. m.–1 p. m.	13.7	119.2	118.9	124.7	133.6	146.8	128.6	104.7	124.1	127.0	127.0	147.2	126.0
	30	1–6 p. m.	13.8	119.1	118.8	124.8	132.7	146.6	128.4	104.0	120.0	126.0	126.1	145.2	124.3
July 10	30	8 a. m.–1 p. m.	15.7	119.0	121.9	124.0	132.2	146.5	128.7	94.6	126.0	129.0	127.4	147.7	125.1
	30	1–6 p. m.	14.7	114.1	122.8	125.7	133.6	146.5	128.5	90.6	116.7	125.2	125.7	146.9	121.0

The split-day treatments usually resulted in a slight reduction in number of tillers and panicles, length of panicles, plant height, and grain and straw weights. The later in the growth period the plants were covered, the greater were the reductions in the characters studied. The effect of the split-day treatments on these characters was not so consistent as it was on the time of heading.

All varieties showed a greater reduction in the number of tillers and panicles from afternoon than from morning light interception, except C. I. 4700 and Nira. The differences in number of tillers and

TABLE 6.—*Average effect on the characters studied of split-day treatments from 8 a. m. to 1 p. m. and from 1 to 6 p. m. as compared with normal-day plots*

Duration of treatment	Sensitive varieties						Less sensitive varieties					
	C. I. 81C	Caloro	Kameji	Improved Blue Rose	C. I. 4700	Average	C. I. 6993	Shoemed	Nira	Fortuna	Rexoro	Average
AVERAGE NUMBER OF DAYS FROM SEEDING TO PANICLE EMERGENCE												
Normal day	107. 1	114. 2	121. 2	131. 0	146. 0	123. 9	85. 5	115. 7	124. 6	124. 8	145. 8	119. 3
8 a. m.–1 p. m.	[1] 114. 2*	119. 4**	122. 9**	131. 8**	146. 4	126. 9	91. 9	122. 4**	127. 2**	126. 4**	146. 7**	122. 9
1–6 p. m.	112. 7	120. 3	124. 1	132. 4	146. 2	127. 1	91. 0	116. 4	124. 7	124. 9	145. 6	120. 5
AVERAGE NUMBER OF TILLERS PER PLANT												
Normal day	4. 61	2. 85	2. 78	3. 17	4. 50	3. 58	2. 55	2. 97	1. 97	1. 91	1. 90	2. 26
8 a. m.–1 p. m.	3. 84	2. 83*	2. 51	2. 77	4. 48	3. 29	2. 60	2. 46	1. 85	1. 82	1. 92	2. 13
1–6 p. m.	3. 81	2. 40	2. 18	2. 63	4. 53	3. 11	2. 44	2. 23	1. 87	1. 70	1. 74	2. 00
AVERAGE NUMBER OF PANICLES PER PLANT												
Normal day	4. 43	2. 73	2. 78	3. 11	4. 50	3. 51	2. 55	2. 84	1. 97	1. 91	1. 90	2. 23
8 a. m.–1 p. m.	3. 76	2. 81*	2. 51	2. 77*	4. 48	3. 27	2. 50	2. 43	1. 83	1. 82	1. 91	2. 10
1–6 p. m.	3. 71	2. 38	2. 18	2. 61	4. 53	3. 08	2. 28	2. 20	1. 86	1. 70	1. 74	1. 96
AVERAGE PANICLE LENGTH (INCHES)												
Normal day	8. 9	8. 4	7. 9	9. 1	10. 2	8. 9	7. 1	10. 5	11. 6	10. 5	10. 8	10. 1
8 a. m.–1 p. m.	8. 4	8. 2	7. 7**	8. 7	10. 1	8. 6**	6. 8	10. 7	11. 3	10. 4	10. 5	9. 9
1–6 p. m.	8. 7	8. 3	8. 1	8. 9	10. 3	8. 9	6. 8	10. 6	11. 2	10. 3	10. 5	9. 9
AVERAGE PLANT HEIGHT (INCHES)												
Normal day	42. 9	39. 4	42. 1	46. 1	54. 0	44. 9	33. 5	43. 0	50. 9	46. 3	46. 8	44. 1
8 a. m.–1 p. m.	42. 2	39. 3	39. 2**	43. 7*	53. 2	43. 5	31. 1	44. 8*	50. 5	46. 6	45. 9	43. 8
1–6 p. m.	42. 2	39. 7	41. 4	44. 8	53. 5	44. 3	32. 4	42. 5	50. 0	46. 2	46. 0	43. 4
AVERAGE GRAIN WEIGHT PER PLANT (GRAMS)												
Normal day	-------	5. 7	-------	7. 5	12. 0	8. 4	------	7. 3	7. 5	7. 2	6. 5	7. 1
8 a. m.–1 p. m.	-------	5. 52	-------	5. 88	11. 23*	7. 5	------	6. 36	5. 98	5. 72	5. 53	5. 9
1–6 p. m.	-------	5. 05	-------	5. 50	12. 29	7. 6	------	5. 08	6. 86	5. 78	5. 91	5. 9
AVERAGE STRAW WEIGHT PER PLANT (GRAMS)												
Normal day	-------	8. 0	-------	15. 5	26. 4	16. 6	------	11. 8	11. 2	8. 8	15. 6	11. 9
8 a. m.–1 p. m.	-------	8. 82	-------	12. 30	27. 13	16. 1	------	10. 10*	9. 74	7. 73	14. 69	10. 6
1–6 p. m.	-------	7. 91	-------	12. 99	28. 58	16. 5	------	8. 61	10. 59	7. 34	13. 53	10. 0

[1] *=Significant differences between treatments from 8 a. m. to 1 p. m. and those from 1 to 6 p. m.; **=highly significant differences between treatments from 8 a. m. to 1 p. m. and those from 1 to 6 p. m.

panicles for Caloro and in number of panicles for Improved Blue Rose appear to be significant. When the eight varieties that showed the greatest reductions from afternoon covering were analyzed as a group, the difference between morning and afternoon covering, both in number of tillers and in number of panicles, seemed highly significant.

The average length of panicle of the five sensitive varieties was shorter when the plants were covered in the morning than when they were covered in the afternoon, and this difference appears to be highly significant for Kameji and for the average of the five varieties. In the less sensitive varieties the differences in length of panicle were not significant, although the panicles of Shoemed, Nira, and Fortuna were somewhat longer from morning than from afternoon light interception.

In plant height, four of the sensitive varieties were shorter from morning than from afternoon covering, and the differences for Kameji and Improved Blue Rose apparently were highly significant. The average difference in plant height between morning and afternoon covering for Caloro, Kameji, Improved Blue Rose, and C. I. 4700 as a group appears to be highly significant. The less sensitive varieties Shoemed, Nira, and Fortuna were taller when covered in the morning than in the afternoon, and as a group the average difference was apparently significant.

Weights of grain and straw per plant were obtained for seven varieties. For C. I. 4700 there was what appears to be a significant decrease in grain weight per plant from morning as compared with afternoon covering. The increase in straw weight for Shoemed from morning as compared with afternoon covering apparently was significant. None of the other varieties showed differences in grain or straw weight, between morning and afternoon covering, that appeared to be significant.

DISCUSSION

In general, the results show that varieties subjected to a daily 10-hour photoperiod usually headed before the controls and that varieties under the split-day treatments headed at about the same time as or later than the controls. Kondo and his associates (*9, 10*), and others have reported similar results with rice in Japan, and Garner and Allard (*4, 5, 6*) with other crops in this country.

The two periods of heading for the sensitive varieties Caloro, Kameji, Improved Blue Rose, and C. I. 4700 were apparently due to the fact that very little tillering had occurred by the time the early covering treatments were concluded. Kondo et al. (*9*) showed that individual tillers of the same plant react independently of one another when subjected to different day lengths. In this study most of the tillers developed after the covering periods ended, so that it is reasonable to assume that the treatments had no effect on the time of heading of such tillers.

The data presented on time of panicle emergence indicate that flower-bud differentiation in the sensitive varieties was initiated by a treatment consisting of a daily 10-hour photoperiod for 10 days beginning as early as 30 days after seedling emergence. On the less sensitive varities, however, except C. I. 6993, this treatment had little if any effect on bud differentiation. This indicates that the less sensitive varieties must reach a certain stage of development before the short-day treatments have any effect on time of flower-bud differentiation.

The reaction to the short-day treatment of the varieties grown appears to be associated with the latitude to which they are native. The sensitive varieties were native to or were developed from varieties native to a latitude above 30° north; the less sensitive varieties were native to a latitude nearer the Equator. The sensitive varieties showed the maximum effect of the short-day treatments on the characters studied for the earliest covering date, with a less marked effect for successively later dates of covering.

The sensitive varieties Caloro and Kameji are of Japanese origin, and Improved Blue Rose probably was selected from a hybrid in which one of the parents was a Japanese variety. C. I. 81C and C. I. 4700 are medium-grain varieties that tiller profusely. These varieties were introduced from the Philippine Islands, but from their reaction to the short-day treatments and other characteristics, they might have been introduced into the Philippines from China. The less sensitive varieties Shoemed, Nira, and Rexoro were also selected from varieties introduced from the Philippine Islands. Fortuna was selected from an introduction from Formosa, and C. I. 6993 is of Japanese origin. As a group, these varieties showed very little effect of the short-day treatments for the earliest covering periods but a more marked effect as the date of covering was delayed.

SUMMARY

Ten varieties of rice, growing under field conditions, were subjected to short-day treatments for 10, 20, and 30 days at different stages of development. The following treatments, in addition to the normal day length, were used: (1) A continuous 10-hour daylight period; (2) approximately 10 hours of daylight with a 5-hour covering period in the morning; and (3) approximately 10 hours of daylight with a 5-hour covering period in the afternoon.

The varieties, based on their reactions to the covering periods, were divided into "sensitive" and "less sensitive" groups. The sensitive varieties, when subjected to a daily 10-hour photoperiod at the earliest covering dates, showed a marked decrease in (1) the number of days required from seeding to first heading, (2) number of tillers and panicles, (3) length of panicle, (4) height of plant, and (5) grain and straw weights. As the date of covering was delayed, the effects became gradually less marked. The less sensitive varieties showed little or no effect on these characters for the earliest covering periods, followed by a gradual intensification of the effect for later covering dates.

Although differences were small, plants subjected to morning and afternoon covering usually headed later than the controls. The sensitive varieties tended to head later from afternoon than from morning covering, while the less sensitive varieties tended to head later from morning covering. The data on length of panicle and plant height were less consistent than for date of heading, but in most cases the sensitive varieties produced the longest panicles and the tallest plants when covered in the afternoon, while the less sensitive varieties produced the longest panicles and the tallest plants when covered in the morning. Both the sensitive and the less sensitive varieties showed a reduction in number of tillers and panicles from afternoon as compared with morning covering. No consistent differences between morning and afternoon covering were recorded for grain and straw weights.

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THE COMPOSITION AND PALATABILITY OF SOME COMMON GRASSES[1]

By J. G. ARCHIBALD, *research professor of animal husbandry*, E. BENNETT, *assistant research professor of chemistry*, and W. S. RITCHIE, *head of Department of Chemistry, Massachusetts Agricultural Experiment Station*

INTRODUCTION

In 1928 a project was organized by the Chemistry Department of the Massachusetts Agricultural Experiment Station entitled, "Studies in the Chemistry of Pasture Grasses." One phase of this project involved a study of the chemical composition of different species of grasses at the grazing stage. This particular study was undertaken because a search of the literature had disclosed a scarcity of information on the chemical composition at the grazing stage of individual species of the grasses commonly grown in the northeastern part of the United States. The study was continued over a period of 7 years (1931–37 inclusive). One progress report (*1*)[2] giving results for the years 1931, 1932, and 1933 has already been published. This paper is the final report and sets forth the results for the entire 7-year period, the earlier published results for 1931, 1932, and 1933 having been combined with those for 1934 to 1937.

Although the chemical composition of grasses has been widely studied, one or more of three circumstances has rendered the findings inapplicable to the present work: (1) The species used differed from those included in this study; (2) the analyses were made when the grasses were too mature to be considered as typical of good grazing, generally at or near the blossom stage; or (3) the studies were made on mixed herbage. A report of a few analyses of *Poa pratensis* by Pammel, Weems, and Lamson-Scribner (*7*), and more recently the work of Grunder (*5*) on *Trifolium repens*, are all that seem to have a direct bearing on this study.

EXPERIMENTAL METHODS

Six species of grasses have been grown as pure or practically pure stands, and their chemical composition has been determined by monthly sampling and analysis throughout the growing season (May–September) of each of the years indicated. In addition, one other species of grass and two species of legumes have been similarly studied during several seasons of the period indicated. Vitamin A assays of the fresh grasses have been made in several seasons, and the palatability of the grasses to cows has been studied by means of actual grazing tests.

Two separate tracts of land were chosen for the investigation. Tract No. 1 was utilized from 1931 to 1936 inclusive for studies of composition, and in 1937 and 1938 for palatability tests. From 1931

[1] Received for publication June 5, 1942. Contribution No. 455 of the Massachusetts Agricultural Experiment Station.
[2] Italic numbers in parentheses refer to Literature Cited, p. 347.

to 1934 inclusive the grasses were grown without fertilizer other than that residual in the soil. In 1934, 1935, and 1936 a complete 8–6–6 fertilizer was applied at the rate of 400 pounds per acre at the beginning of the growing season.

Tract No. 2 was seeded in 1933, but samples were not taken until 1935 because flooding and winter-killing had necessitated complete reseeding. The analytical studies were continued through 1935, 1936, and 1937, and in 1938 palatability tests were conducted. Fertilizer was not applied to this set of plots at any time during the investigation. Analyses of the soils of both tracts are given in table 1; the principal differences are the somewhat larger amount of fine material and the very much larger amounts of organic matter and nitrogen in the soil of tract 2.

TABLE 1.—*Analysis of the air-dry soils* [1]

Tract and soil profile	Fine soil (1 mm or less)	Organic and volatile matter	Total nitrogen	Total phosphorus	Total potassium	Total calcium	Available phosphorus	pH
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Parts per million*	
Tract 1:								
Surface soil	92.17	5.05	0.14	0.18	2.01	1.43	154	6.05
Subsoil	99.33	2.63	.04	.03	1.85	1.44	7	5.50
Tract 2:								
Surface soil	98.50	12.87	.41	.12	1.86	1.17	73	5.52
Subsoil	90.90	2.89	.06	.03	2.35	1.44	11	5.65

[1] All determinations except available phosphorus were made by the methods of the Association of Official Agricultural Chemists (*2*). Available phosphorus was determined by Truog's method (*8*).

The grasses and legumes chosen for the investigation were: Kentucky bluegrass (*Poa pratensis*), also called Junegrass; orchard grass (*Dactylis glomerata*); redtop (*Agrostis alba*); Rhode Island bent (*Agrostis capillaris*), also called colonial bent; timothy (*Phleum pratense*), also called herd's grass; sheep fescue (*Festuca ovina*); sweet vernal grass (*Anthoxanthum odoratum*); white Dutch clover (*Trifolium repens*); and Ladino clover (*Trifolium repens*).

Each series of plots was replicated once, making a total of 16 plots in the first series and 18 in the second. Each plot was 3½ feet wide by 62 feet long, and had an area of approximately one two-hundredth of an acre. To prevent mixing of the species and to facilitate control of weeds, paths 1 foot wide were maintained between individual plots and a border of fallow soil 3 feet wide was left along the margins of each tract. Seed was sown in the late summer (except for a few cases of reseeding) without a nurse crop and the plots were kept free from weeds by hand labor. A reasonable degree of success was attained in keeping pure the stands of the individual species.

Samples were taken by means of a lawn mower with a grass catcher attached whenever the grass reached a height of 3 to 4 inches, so that intervals between samplings varied with species and season of the year. Whenever possible at least one sample a month was taken from each species; wherever rate of growth necessitated taking more than one sample in a month from any species, yield and moisture content were determined on the additional samples, and each sample was composited with the sample already taken from the same plot.

Analyses were made for moisture in the fresh grass, and for total nitrogen, crude fiber, ether extract, total ash, acid-soluble ash, calcium phosphorus, and magnesium in the dry matter. Potassium was determined in the samples for 1931, 1932, and 1933, but because of its minor significance in the nutrition of grazing animals, it was not determined from that time onward.

All analyses except those for calcium, phosphorus, and magnesium were made according to the official methods of the Association of Official Agricultural Chemists (*2*).[3] Calcium and magnesium were determined by a modification of McCrudden's method (*6*), and phosphorus by the method of Fiske and Subbarow (*4*). Vitamin A was determined by biological assay with white rats, on a much smaller number of samples (51) taken for the most part independently of the main investigation.

During May and June of 1937 and 1938 a series of tests of the palatability of seven of the species was conducted with mature Jersey and Guernsey cows in milk at the time. The entire tract of each series of plots was fenced off, the plots were conspicuously numbered, and a tub of water was placed at a convenient point within the enclosure. Two cows were used at a time. They were turned into the enclosure soon after 8 a. m. and taken out again shortly before noon. There was sufficient grass so that each trial extended over 2 such half days.

A complete record of the length of time spent by each cow in grazing each species was obtained by an attendant who noted the exact time that a cow started and stopped grazing on any particular plot. Lulls in grazing, after which the cow resumed grazing on the same plot, were recorded as carefully as shifts from plot to plot.

PRESENTATION OF RESULTS

CHEMICAL ANALYSES

A total of 300 samples of the several species was taken, but this number was reduced for analysis to 263 by the compositing of certain samples.

The results on the first six species listed in table 2 form the principal basis for discussion. Such information as was obtained on the last three species has been appended as a supplement to the more complete report on the first six. There are two reasons for this procedure: (1) The much larger number of samples from individual species in the first group, and (2) each and every year and a majority of months are represented in the data from the first group, whereas for unforeseen or unavoidable reasons this was not true for any of the three species in the second group.

In this study there were representatives of two great classes of herbage, the so-called top and bottom grasses, or more specifically those grasses more suitable for hay as contrasted with those more suitable for grazing. Some interesting differences are apparent in the composition of the species in these two classes. The top grasses were decidedly more succulent than the bottom grasses; they averaged about 2.5 percent less fiber and contained larger amounts of

[3] Grateful acknowledgment is made of the services of J. W. Kuzmeski and A. F. Spelman, who made the determinations of crude fiber and ether extract under the direction of Philip H. Smith, chief chemist of the feed control laboratory of this station.

ether extract, soluble ash, calcium, magnesium, and vitamin A (carotene). These differences probably are due in considerable measure to differences in stage of maturity and relative leaf area of the two classes of grass at a given height; they have a bearing on grazing practice that should not be lost sight of.

TABLE 2.—*Composition of 7 species of grass and 2 legumes, seasons of 1931–37*

Species	Samples	Moisture in the fresh grass	Composition of dry matter								Vitamin A equivalent per pound of dry matter
			Nitrogen[1]	Crude fiber	Ether extract	Total ash	Acid-soluble ash	Calcium	Phosphorus	Magnesium	
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*International units*
Top grasses:											
Orchard grass	34	76.2	2.9	23.0	4.5	12.7	8.4	0.63	0.58	0.32	152,000
Redtop	36	74.0	3.0	21.9	4.2	10.6	6.8	.68	.39	.27	133,000
Timothy	36	74.2	2.9	20.6	3.9	9.5	6.3	.59	.40	.25	162,000
Bottom grasses:											
Kentucky bluegrass	39	69.6	3.0	23.6	3.4	9.0	5.8	.51	.42	.23	101,000
Rhode Island bent	37	70.6	3.1	21.7	3.7	10.3	6.2	.64	.40	.23	132,000
Sheep fescue	39	67.1	2.7	26.8	3.3	8.4	5.0	.43	.36	.16	85,000
Sweet vernal grass	14	77.9	3.2	17.6	4.7	10.0	6.8	.64	.38	.27	118,000
White Dutch clover	20	82.6	4.6	14.4	3.1	12.3	9.9	1.61	.45	.29	(2)
Ladino clover	8	83.7	4.3	13.7	2.5	13.5	8.8	1.90	.37	.42	(2)

[1] Reported as nitrogen rather than as protein because not all the nitrogen is in the form of protein; the amount of nonprotein nitrogen is small—10 percent or less.
[2] Not determined.

Nitrogen differences between species were small for the most part, and the same was true of phosphorus. The high value for phosphorus in orchard grass noted in the earlier report (*1*), while still persisting in the samples grown on soil of tract 1, was not noted in the samples from soil of tract 2. This may be due to the fact that there was about twice as much available phosphorus in the soil of tract 1 as in that of tract 2 (see table 1). It has been suggested that possibly the ability to utilize additional phosphorus may be a characteristic peculiar to to this species of grass.

There seems to be a rather definite relation between the amount of fiber in the several species and their breaking strength. Beaumont, Stitt, and Snell (*3*), of this station, have reported breaking strength for four of the six species represented here. Their values, arranged in order of increasing strength, are compared with those for fiber content in table 3. Except that the timothy had a little less fiber than the

TABLE 3.—*Breaking strength compared with fiber content of four of the species of grasses included in the test*

Species	Breaking strength [1]	Fiber content
	Grams	*Percent*
Redtop	52.9	21.9
Timothy	58.6	20.6
Kentucky bluegrass	92.6	23.6
Sheep fescue	147.9	26.8

[1] Grams per millimeter of perimeter; average of 50 determinations.

redtop, the two sets of values are in the same order; i. e., the higher the content of fiber the higher the breaking strength, which is what would naturally be expected.

The proportion of soluble ash to total ash was rather constant (table 2) the variation being only from 66.2 percent in orchard grass to 59.4 percent in sheep fescue; although there were some exceptions, in general the higher the percentage of soluble ash the greater the proportion of the total ash it constituted. As in the earlier trials (*1*), the two members of the bent family (redtop and Rhode Island bent) had the highest calcium values of the group, but contrary to earlier results, the value for redtop was higher than that for Rhode Island bent.

In general, the constitutents that varied significantly from one species to another were: Moisture, in the fresh grass; and, in the dry matter, crude fiber, ether extract, soluble ash, calcium, and magnesium. With minor exceptions phosphorus and nitrogen did not vary significantly.

In addition to the six species just discussed, three other species—one grass and two legumes—were studied, but in less detail, as already noted. The data are too incomplete to warrant statistical analysis, but they are given at the bottom of table 2 for such information as they may furnish.

In the limited number of samples available sweet vernal grass averaged higher in moisture, nitrogen, and ether extract, and lower in crude fiber than any of the other six species of grass. It was also quite high in soluble ash and magnesium, and was in a class with redtop and Rhode Island bent in its content of calcium.

White Dutch clover was decidedly more succulent than any of the grasses, its nitrogen (protein) content, like that of all legumes, was high (equivalent to 27 percent of protein in the dry substance); its fiber content was about 7 percent less than that of timothy, the best grass in this respect; its soluble-ash content of almost 10 percent was approached only by that of orchard grass; its calcium content was nearly 2½ times as much as that of redtop, the grass with the highest calcium value; its phosphorus content was exceeded only by that of orchard grass, and the same was true for magnesium. Only in ether extract was it lower than the grasses, containing less than sheep fescue, the lowest grass in this respect.

The small number of samples of Ladino clover (a giant variety of *Trifolium repens*) show its composition to be very similar in most respects to that of the dwarf white Dutch variety.

STATISTICAL ANALYSES

In the course of the statistical studies some interesting correlations were observed; those of special significance were between:

Moisture and nitrogen	$r=0.59\pm0.03$
Ether extract and soluble ash	$r=.55\pm.03$
Soluble ash and phosphorus	$r=.74\pm.20$
Calcium and magnesium	$r=.70\pm.02$

There was one significantly negative correlation, that between nitrogen and crude fiber, $r=0.62\pm0.03$.

PALATABILITY TRIALS

Palatability trials with five cows extended over 105 hours of potential grazing time and 54 hours of actual grazing time. A summary of the results of these trials, classified according to individual preference for the several species of grass, as well as the average preference for the entire series of trials, is presented in table 4. The cows showed a decided preference for timothy. In order of preference orchard grass, redtop, Rhode Island bent, and sweet vernal grass ranked so close together (16 to 17.5 percent) that probably the small differences between them were not significant. Bluegrass, however, was grazed less than 10 percent of the time, and sheep fescue was practically untouched.

Separation of the results into groups for first and second day, for first, second, and third trials, and for the two series of plots shows that the trends of preference noted for the results as a whole persisted remarkably all through these smaller groups.

Some variation in individual preference was noted, but the general trend was unmistakable, especially as to the best-liked and least-liked species. In palatability timothy was at or near the top; redtop was near the top; bluegrass was near the bottom—not higher than fifth place in any case—and fescue was consistently in last place.

TABLE 4.—*Summary of results of the grazing trials classified according to individual preference for the several species*

Cow No.	Total grazing time	Kentucky bluegrass		Orchard grass		Redtop		Rhode Island bent		Sheep fescue		Sweet vernal grass		Timothy	
		Grazing time	Proportion of total	Grazing time	Proportion of total	Grazing time	Proportion of total	Grazing time	Proportion of total	Grazing time	Proportion of total	Grazing time	Proportion of total	Grazing time	Proportion of total
	Min.	*Min.*	*Pct.*	*Min.*	*Pct.*	*Min.*	*Pct.*	*Min.*	*Pct.*	*Min.*	*Pct.*	*Min.*	*Pct.*	*Min.*	*Pct.*
760	787	90	11.4	142	18.0	151	19.2	147	18.7	3	0.4	80	10.2	174	22.1
806	442	38	8.6	78	17.7	98	22.2	65	14.7	1	.2	83	18.8	79	17.9
607	198	15	7.6	46	23.2	25	12.6	21	10.6	0	----	49	24.8	42	21.2
665	863	28	3.2	105	12.2	112	13.0	131	15.2	15	1.7	217	25.1	255	29.6
687	950	145	15.3	196	20.6	166	17.5	159	16.7	7	.7	89	9.4	188	19.8
Total	3,240	316	----	567	-----	552	-----	523	-----	26	----	518	-----	738	-----
Weighted average	------	------	9.8	------	17.5	------	17.0	------	16.1	------	.8	------	16.0	------	22.8

On the basis of the preference shown by the cows as a group the rating of the grasses is without exception in the order of their vitamin A (carotene) content, and with one exception in the order of their succulence (moisture content). In general also the cows preferred those with a relatively high content of ether extract, soluble ash, and magnesium, and with a low content of fiber. Nitrogen (protein) content of the grasses apparently had little if any relation to their palatability.

The observation of some investigators that redtop lacks palatability does not agree with the rating it received in these tests. The discrepancy probably is due to the fact that in these trials it never reached the stage where it developed woody stolons.

SUMMARY

This is the final report of a 7-year investigation into the chemical composition and palatability to cows of certain grasses and legumes grown as practically pure stands. The species studied were: Kentucky bluegrass, orchard grass, redtop, Rhode Island bent, sheep fescue, timothy, sweet vernal grass, white Dutch clover, and Ladino clover.

In the main investigation 300 samples, reduced by compositing to 263, were analyzed. In addition, 51 biological assays were made for vitamin A, and palatability tests with 5 cows extended over 105 hours of potential grazing time and 54 hours of actual grazing time.

Considerable species differences were noted in the several constituents except nitrogen and phosphorus [4]; these differences for the most part were more obvious between the two groups of grasses (top and bottom) than between the individuals within a group. Relatively the greatest group differences were in vitamin A (carotene), magnesium, soluble ash, and ether extract, a reflection probably of greater leaf area in the top grasses. The composition of the two legumes was in rather sharp contrast to that of the grasses. Highly significant correlations were noted between certain of the constituents.

Cows used in palatability tests showed a definite preference for species high in moisture and carotene and low in fiber. The nitrogen (protein) content of the grasses apparently had little if any relation to their palatability.

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[4] One species (orchard grass) had a much higher phosphorus content than the others under certain circumstances.

THE PROTEINS OF VARIOUS TREE SEEDS[1]

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INTRODUCTION

The fact that acorns, ironwood seed and elm seed are used as food by wild animals, and to some extent by domestic animals, has prompted this investigation to determine their protein content. Several feeding experiments have been conducted with acorns, but, so far as the writers are aware, no study has been made of the nitrogenous constituents, particularly the protein fractions, of any of these typical tree seeds.

This paper describes a peptization study of the proteins of ironwood seed, elm seed, and several varieties of acorns, the isolation of their major protein fractions, and a study of these proteins according to the method of Van Slyke (*10*).[2]

MATERIALS AND METHODS OF EXPERIMENTATION

The varieties of tree seeds studied are listed according to the classification of Rosendahl and Butters (*7*).

- Beech family:
  - Black oaks:
    - Northern red oak (*Quercus borealis*).
    - Red oak (*Quercus borealis* var *maxima*).
    - Northern pin oak (jack oak) (*Quercus ellipsolidalis*).
  - White oaks:
    - White oak (*Quercus alba*).
    - Northern bur oak (*Quercus macrocarpa* var. *olivaeformis*).
- Elm family:
  - American elm (*Ulmus americana*).
- Birch family:
  - Hop-hornbeam or ironwood (*Ostrya virginiana*).

Collections of the various seeds were made during the seasons of 1933, 1936, and 1937, and the material was dried at room temperature. The husks or shells were removed and the seeds ground in a burr mill. The meal was treated in a Soxhlet extractor with naphtha gasoline to remove the fat. The extracted seed meal was dried in an oven at 55° C. and at atmospheric pressure, and further ground in a ball mill until all the material passed through a 100-mesh sieve.

The fat-extracted material will be referred to in this paper as the seed meal. The percentage of fat was determined in the Bailey-Walker apparatus with ether as a solvent. The seed meal was analyzed for moisture and ash by standard methods. Total nitrogen was determined on the seed meal by the Kjeldahl-Gunning-Arnold method, and calculated on the ash- and moisture-free basis.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 355.

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EXPERIMENTAL DATA

PEPTIZATION STUDIES ON THE SEED PROTEINS

METHODS

The methods described for this division of the experimental work have been developed on the basis of the general classification given for proteins.

A. Duplicate 2-gm. samples of seed meal were weighed out and transferred to 100-ml. pyrex centrifuge tubes and suspended in 25 ml. of distilled water. This was stirred with a glass rod for 20 minutes and then centrifuged until the liquid was clear. The clear peptizates were decanted into 800-ml. Kjeldahl flasks. A second and a third successive extraction were made in the same manner and the three peptizates were combined for the determination of total nitorgen. It is recognized that salts were present in the meals and may have slightly altered the peptization values. Since the concentrations of these salts in the water range from 0.58 to 0.18 percent as calculated from the ash determination, and since the procedure detailed in paragraph B results in additional protein, the results obtained here are reported as the water-soluble nitrogen fraction.

B. The residues in the 100-ml. centrifuge tubes, after the water-soluble protein had been removed, were next extracted with a 5-percent solution of C. P. potassium chloride in distilled water. This solution has a pH value of 6.78. Three extractions were made according to the procedure described in paragraph A. This is reported as the nitrogen soluble in saline solution.

C. The residues in the 100-ml. centrifuge tubes from paragraph B were extracted with 70-percent alcohol at 65° to 70° C., according to the procedure described in paragrahp A. This is reported as the alcohol-soluble nitrogen.

D. The residues, after fractions A, B, and C of proteins had been removed, were extracted with a 0.2-percent solution of potassium hydroxide. Three extractions were made exactly as described in paragraph A. This fraction is reported as the alkali-soluble nitrogen.

E. The nitrogen in the residues from paragraph D was determined by the Kjeldahl-Gunning-Arnold method and is listed as the residual nitrogen.

RESULTS

The results of the analyses of the seeds made according to the methods outlined above, are given in table 1.

TABLE 1.—*Analysis of the ground tree seeds of various crops*

Seed crop	Fat in dry, ground seed	Seed meal			Proportion of total nitrogen [2]—					Total nitrogen recovery
		Moisture [1]	Ash [1]	Total nitrogen [2]	Water-soluble	Soluble in saline solution	Alcohol-soluble	Alkali-soluble	Residual	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Elm, 1936	33.55	6.70	7.30	11.55	35.30	46.30	0.70	12.50	5.60	100.40
Ironwood:										
1933				3.17	38.35	35.92	2.28	8.82	13.87	99.24
1937	19.21	6.00	4.65	2.69	12.43	15.94	4.27	38.58	29.72	100.94
White oak, 1936	7.94	3.05	2.65	2.14	21.31	2.58	4.64	40.83	31.29	100.65
Northern bur oak, 1936	7.86	5.80	2.93	1.68	28.62	2.81	5.24	42.98	18.71	98.40
Northern red oak, 1937	20.56	5.18	3.48	1.47	9.85	1.74	6.56	56.22	25.71	100.08
Red oak, 1937	11.60	4.53	3.33	1.52	15.98	1.38	5.84	34.98	41.48	99.66
Northern pin oak, 1937	20.00	5.02	2.22	2.09	6.68	2.41	6.15	58.77	26.31	100.32

[1] Fat-free basis. [2] Fat-, moisture-, and ash-free basis.

ISOLATION OF PROTEINS FROM THE PREPARED SEED MEAL

Methods

The protein in the seed meal from the elm seed, ironwood seed, white oak, northern bur oak, and northern red oak was fractioned into albumin, globulin, prolamin, and glutelin by peptization in exactly the same manner as described in paragraphs A, B, C, and D above. Ten-gram samples of seed meal, 250-ml. centrifuge tubes, and 250-ml. portions of extracting solutions were used.

A. For the isolation of the albumin fractions from the different meals, the pH of minimum solubility was determined. This was made by the addition of varying amounts of 50 percent acetic acid to a series of tubes of peptizates (pH range from 5.4 to 2.7), and the solutions were boiled for 20 minutes. The material was centrifuged and the unprecipitated nitrogen was determined on aliquots of the clear liquid by the Kjeldahl method. The pH at which the precipitation was most complete was taken as the pH of minimum solubility.

Large volumes of the albumin peptizates were adjusted to the pH of minimum solubility, the protein was coagulated, and then separated from the mother liquors by centrifugation. The proteins were dried with 65-percent, 75-percent, 85-percent, and 95-percent alcohol, used in turn, and finally with anhydrous acetone. The acetone was removed by drying the preparations in an oven at 55° C.

B. The globulins were precipitated from the salt peptizates by dialysis in cellophane tubes against tap water for 12 hours and finally against distilled water for about 24 hours. The proteins were separated from the mother liquors and dried as described in paragraph A.

C. The alcohol extract was diluted with a large volume of water containing a little sodium chloride. A small precipitate resulted which was again dissolved in aqueous alcohol and precipitated. The yield was not enough to permit further study.

D. The pH at which the glutelins were most completely precipitated from the alkaline extracts was determined in the same manner as for the albumin described in paragraph A, except that heating was not necessary. The pH of minimum solubility of the protein is given in table 2. Large volumes of peptizates were adjusted to the pH of

TABLE 2.—*Minimum solubility of proteins*

Seed meal	Protein	pH range	Seed meal	Protein	pH range
Ironwood	Albumin	3.9–3.4	Northern red oak	do	4.4–3.5
	Glutelin	4.2–3.5	Elm	Albumin	3.8
White oak	Glutelin	4.7–3.9		Glutelin	4.5–3.7
Northern bur oak	do	4.3–3.6			

minimum solubility of the protein by the addition of 50-percent acetic acid and the protein separated from the mother liquors and dried according to the method described in paragraph A.

E. No attempt was made to study the nature of the nitrogen in the residue.

Results

Elm seed and ironwood seed.—The albumin, globulin, and glutelin fractions behaved normally when the above-described methods of

isolation were employed, but the prolamin fractions were so small that their isolation would have been difficult, if not impossible.

Acorns.—The major protein, glutelin, was isolated from white oak, northern bur oak, and northern red oak by the method described in paragraph D. All yields are given in table 3.

TABLE 3.—*Protein isolation yields from various seed meals*

Seed meal	Fraction	Protein yield	Seed meal	Fraction	Protein yield
		Percent			*Percent*
Ironwood:			Elm:		
1933	Albumin	1.61	1934	Albumin	3.65
	Globulin	2.00	1936	do	7.00
	Glutelin	1.17		Globulin	24.30
1937	Albumin	1.22		Glutelin	13.40
	Globulin	.90	Northern bur oak, 1936	do	8.02
	Glutelin	9.02	White oak, 1936	do	8.44
			Northern red oak, 1936	do	5.56

Attempts to precipitate albumin or globulin fractions from the peptizates of the acorn meals by the method described in paragraphs A and B failed. A 5 percent solution of the trichloracetic acid also failed to yield precipitates.

The alcohol-soluble proteins from the acorns precipitated very slowly by the method described in paragraph C above. The yield of protein was very low. Five hundred grams of northern red oak seed meal yielded 1.62 gm. of prolamin which contained 6.18 percent nitrogen. Acorns contain a large amount of alcohol-soluble gums that precipitate with the protein.

The water- and the salt-soluble extracts corresponding to the albumin and globulin fractions of the acorns were studied according to the procedure used by Hiller and Van Slyke (*4*), with tungstic acid as a precipitant. The peptizate from 20 gm. of seed meal was used in this study. The data obtained are given in table 4.

TABLE 4.—*Analysis of the albumin and globulin in acorns of three species of oak trees*

Tree species	Fraction	Total nitrogen in peptizate	Total nitrogen in filtrate	Nitrogen precipitated by tungstic acid	Amino nitrogen in peptizate
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
White Oak	Albumin	85.29	83.47	1.82	24.63
	Globulin	9.84	1.46	8.38	
Northern bur oak	Albumin	93.54	88.75	4.79	21.42
	Globulin	9.56	4.98	4.58	
Northern red oak	Albumin	20.15	16.69	3.46	17.20
	Globulin	4.69	0	4.69	

ANALYSIS OF THE PROTEINS ISOLATED FROM THE TREE SEEDS

Moisture, ash, and total nitrogen were determined by the usual technique. The percentages of nitrogen were calculated on the moisture- and ash-free basis.

A nitrogen-distribution study was made according to the method of Van Slyke (*10*) as modified by Plimmer and Rosedale (*6*). *l*-Cystine determinations were made on the basic fraction and on the filtrate

from the bases by the method of Sullivan and Hess (*8*, *9*). Tyrosine and tryptophane were determined on a tenth of a gram of protein by the method developed by Folin and Ciocalteu (*2*) and modified by Folin and Marenzi (*3*) and Da Silva (*1*). The data from this experiment are given in table 5. All analyses were made in duplicate but only the averages are reported here.

TABLE 5.—*Nitrogen distribution of the proteins of various tree seeds in percentage of total nitrogen, calculated on a fat-, ash-, and moisture-free basis*

Distribution of nitrogen [1]	Ironwood			Elm			White oak	Northern bur oak	Northern red oak
	Albumin	Globulin	Glutelin	Albumin	Globulin	Glutelin	Glutelin	Glutelin	Glutelin
Acid-insoluble humin nitrogen	1.33	0.67	3.35	2.15	1.18	2.40	3.16	4.34	3.19
Acid-soluble humin nitrogen	.67	.26	.32	.52	.34	.17	1.87	1.44	1.43
Phosphotungstic acid humin nitrogen	1.30	1.07	1.17	1.94	.98	.81	2.41	1.77	2.15
Amide nitrogen	10.67	10.69	11.25	7.84	10.63	8.67	10.86	9.78	11.80
Total nitrogen of the filtrate	51.35	52.75	49.66	50.95	47.71	51.04	51.87	54.54	54.37
Total nitrogen of the bases	34.81	35.29	34.48	36.49	39.29	36.61	29.94	28.46	27.01
Recovery	100.13	100.73	100.23	99.89	100.13	99.70	100.11	100.33	99.95
Amino nitrogen of the filtrate	45.36	46.72	44.98	34.50	41.00	44.88	47.10	49.76	35.71
Nonamino nitrogen of the filtrate	5.99	6.03	4.68	16.45	6.71	6.16	4.77	4.78	18.66
Amino nitrogen of the bases	13.24	13.26	12.08	16.28	14.80	14.94	13.04	12.52	11.37
Nonamino nitrogen of the bases	21.57	22.03	22.40	20.21	24.49	21.67	16.90	15.94	15.64
Arginine	27.97	27.38	27.14	22.81	29.61	28.63	19.20	16.65	18.24
Histidine	.89	2.25	3.07	4.65	3.28	5.52	3.75	5.19	2.96
Cystine:									
Bases	.14	.10	.12	.78	.58	.73	.39	.24	1.16
Filtrate	.40	.35	.32	1.05	.84	.88	.43	.43	1.24
Lysine	5.80	5.57	4.17	8.24	5.36	6.74	6.59	6.38	4.66
Tyrosine	1.11	1.21	1.12	1.82	.10	1.63	1.75	1.93	1.83
Tryptophane	.48	.82	1.44	.48	.53	1.52	1.69	1.94	1.25
Moisture	7.70	7.42	2.28	3.85	7.00	4.91	9.70	8.90	6.20
Ash	6.20	1.86	1.10	5.72	.36	1.61	2.32	1.64	2.15
Nitrogen	16.94	18.54	12.61	15.53	19.19	14.80	13.04	12.83	10.74

[1] Determined by the method of Van Slyke (10) as modified by Plimmer and Rosedale (*6*).

DISCUSSION

PEPTIZATION

The peptization study on ironwood seed (table 1) suggests that there is a great difference in the nature of the protein laid down in the seed during a dry year (1933) and during a year when rainfall is normal (1937). The seed collected during the dry year of 1933 had a slightly higher total nitrogen content than the seed collected in 1937. This observation is in harmony with the findings of cereal chemists in studies of cereal grains, particularly wheat. The study further revealed that the crop of 1933 had a much larger water-soluble and salt soluble protein fraction, a correspondingly small alkali-soluble protein fraction, and less residual nitrogen than the crop of 1937. Since ironwood seed ripens in September climatic conditions in August and September must be considered in predicting the nigtrogen content of the seed.

The analyses of acorns from five varieties of oaks reveal that the seed of the red oak is much richer in fat than that of the white oak. The nitrogen content of all the different varieties of acorns is low

and the residual nitrogen accounts for from 18 to 40 percent of the total nitrogen. The major protein in acorns is a glutelin.

PROTEINS ISOLATED

Tungstic acid will precipitate proteins, peptones, and proteoses (*4*). An examination of table 4 with this in mind shows that there is very little protein nitrogen in the water-soluble (albumin) fraction from acorns and that a greater part of the nitrogen in the salt soluble fractions is protein in nature. Acorns are rich in alkali-soluble gums that precipitate with the protein, resulting in a product low in nitrogen.

VAN SLYKE ANALYSIS OF THE PROTEINS

An outstanding difference between the red oak and the two white oak proteins lies in the high nonamino nitrogen in the filtrate from the bases of the red oak. This includes the proline, oxyproline, and tryptophane nitrogen of the protein. The nitrogen distributions of the three protein fractions from ironwood seed are very much alike, while a similar comparison of the protein fractions of elm seed reveals a considerable difference.

The arginine content of the proteins examined is notably high and the arginine nitrogen represents the major percentage of the total basic nitrogen. These values range from 77.6 to 80.4 percent for the ironwood seeds, from 62.5 to 78.2 percent for the elm seeds, and from 58.5 to 67.5 percent for the acorns. Larmour (*5*) has collected the analyses of a variety of plant proteins; of these edestin is recognized as being rich in arginine with 81.6 percent of the basic nitrogen in this form, whereas the various cereal proteins contain 45 to 65 percent of the total basic nitrogen as arginine nitrogen. In this respect, as well as in the other constituents determined, the tree-seed proteins appear to be within the range reported for other edible-seed proteins.

SUMMARY AND CONCLUSIONS

The percentage of fat, ash, and total nitrogen in the following tree seeds has been determined: American elm, ironwood, white oak, northern bur oak, northern red oak, red oak, and northern pin oak.

The acorns from red oaks are richer in fat than the acorns from white oaks. Elm and ironwood seeds are rich in fat.

Acorns are low in protein, ironwood seeds are somewhat higher in protein, while elm seeds are very high in protein.

A peptization study on the proteins of the above-named tree seeds has been made. The water-soluble, saline-solution-soluble, alcohol-soluble, alkali-soluble, and residual nitrogen have been determined on the same sample of each seed meal.

The major proteins have been isolated from the seeds studied. From the American elm there were obtained an albumin, a globulin, and a glutelin. The same types of protein were isolated from ironwood seeds. From the three oaks only the glutelin fraction was obtained.

The above-named proteins have been analyzed for nitrogen distribution according to the method of Van Slyke (*10*).

Tyrosine, tryptophane, and cystine have been determined colori-

metrically. The last-named was determined on both the basic fraction and the filtrate from the bases.

The ratio of arginine nitrogen to total basic nitrogen is strikingly high, but in this respect is similar to that of other seed proteins.

The water- and salt-soluble nitrogen fractions from acorns of white oak, northern bur oak, and northern red oak have been studied with tungstic acid as a precipitant. The water-soluble fraction was chiefly nonprotein in nature while the saline-solution-soluble fraction was chiefly protein.

Acorns contain large amounts of alkali-soluble gums that interfere with the isolation of the major protein fractions. Eighteen to forty percent of the nitrogen is residual nitrogen.

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SOME METHODS FOR APPROXIMATE PREDICTION OF SURFACE AREA OF FRUITS[1]

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INTRODUCTION

It is sometimes desirable to know the surface areas of apples that are to be used in investigations relating to spray coverage, spray-residue removal, respiration, or coloration. This paper presents the results of a study to determine whether satisfactory mathematical equations could be developed that would facilitate approximate predictions of exposed surfaces or surface areas of different varieties and kinds of fruits at different stages of growth or maturity.

MATERIAL AND PROCEDURE

During the fall of 1940 Johathan, Delicious, and McIntosh apples were obtained from orchards of the Michigan Agricultural Experiment Station for the purpose of determining surface areas of apples and making certain measurements that might be correlated with surface area. Jonathan apples were picked at random on September 5, September 20, and October 24; Delicious apples were harvested on September 14, September 28, and October 22; McIntosh apples were gathered when they reached maturity. Seventy-five apples were taken at each sampling period. Stayman and a second sample of McIntosh apples were bought in a store in Lansing, Mich., in December. Each apple was cut through the middle transversely as shown by a cross section in figure 1. Narrow strips of peel were then removed, pinned to a sheet of paper, and traced with a sharp pencil (fig. 1). The areas of each strip and each cross section were found by means of a planimeter, carefully manipulated by one person. The measurements made on Jonathan apple No. 2, harvested September 5, are shown in figure 1. Preliminary investigations with two samples, each of 100 apples from the 1939 crop, indicated that 75 fruits were enough to use for obtaining surface area with an accuracy of 5 percent of the mean.

After several trials it was found that the peeling should be cut into narrow strips (approximately three-fourths inch at the widest point) because when wide strips were used, the peelings wrinkled and were very difficult to trace accurately. The strips were between one-eighth and one-sixteenth inch thick. At this thickness the peeling will not curl at the edges when being traced and the pencil can move around the edges with little difficulty. Each strip was

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fastened to the pad with several pins to prevent buckling; this held the peeling in position and flattened it on the paper. The pieces of peeling were more or less irregular at the ends because of the shapes of the basins and cavities of apples. A little care enabled the peeler to cut so as to remove the entire peeling to the bottom of the cavity or the basin.

Some slight errors were made in tracing and measuring areas with a planimeter, but careful work reduced these to very small amounts. The planimeter was read to 0.01 of a square inch. Any strip of peel, regardless of width, is convex or concave, depending on the side it is viewed from, and cannot therefore be made to lie completely on a flat surface. To secure an estimate of the amount of error caused by curvature, several pieces of tissue paper of different shapes (triangular, rectangular, and square) whose areas were known, were glued to various parts of the surface of an apple before it was peeled. These were cut from the apple with the peeling, pinned onto a sheet of paper, and traced. The areas of these tracings were found with the pla-

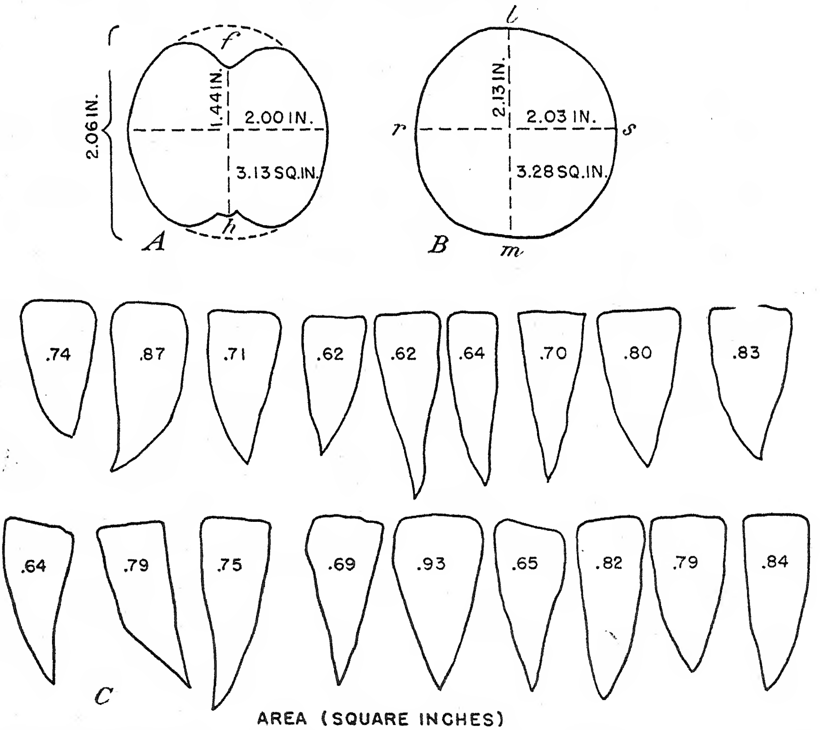


FIGURE 1.—Diagrams showing axial cross section (*A*), transverse cross section (*B*), and narrow strips of peel (*C*) removed from apple and pinned to a sheet of paper for tracing to determine the area with a planimeter. Measurements given are those of Jonathan apple No. 2, harvested September. 5.

nimeter. In several instances, the planimeter areas of these tracings were the same as the original areas of the tissue paper; in the others, the planimeter areas differed from the known areas by one or two points in the second decimal place. This would indicate that the

small strip-planimeter method will give good results, and that the sum of the areas of the peeling strips from an apple should be approxmately equal to the surface area of the apple.

Various methods have been employed for measuring the external surface of apples. Russell and Marshall [2] nailed one-half of an apple to a table, cheek facing the table and periphery of the cut surface marked with an indelible pencil, and then by tissue paper brought up around the half, estimated the surface area of half of the apple and then multiplied this by two to secure the entire surface of the apple. This method would appear to be difficult to use as the paper folds and wrinkles when brought up around the convex surface. Again, it is not easy to cut unsymmetrical apples into two equal parts.

SURFACE-AREA DETERMINATIONS FROM SURFACE OF ELLIPSOID [3]

The surface area of an ellipsoid with axes $a>b>c$, similar to the one shown in figure 2, is, according to Wilson, [4]

$$(1) \qquad 2\pi C^2+\frac{2\pi ab}{\sin\phi}\left[\frac{c^2}{a^2}F(\phi,k)+\frac{a^2-c^2}{a^2}E(\phi,k)\right], \text{ where } \cos\phi=\frac{c}{a},$$

$$k=\frac{b^2-c^2}{b^2\sin^2\phi}, F(\phi,k)=\int_0^\phi\frac{d\theta}{\sqrt{1-k^2\sin^2\theta}} \text{ and}$$

$$E(\phi,k)=\int_0^\phi\sqrt{1-k^2\sin^2\theta}\,d\theta.$$

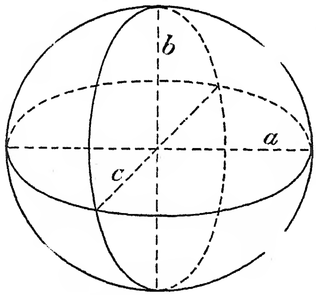


FIGURE 2.—Outline of an apple when it is considered as an ellipsoid with diameters, (a, b, c) equal to those of the McIntosh apple shown in figure 3, *C.*

To apply this formula to apples, it is necessary to show that apples may be considered as ellipsoids with some adjustments. If one were able to pull the peeling out from the stem cavity and from the basin, as is partly shown by dotted lines in figure 3, an object similar to an ellipsoid would result. The surface of the apple has not been changed, for the area of the skin in the cavity and the basin is the same after it is pulled out as before. With a little care one can draw the dotted lines so that the curvature at the points a and b, c, and d in figure 3

[2] RUSSELL, C. E., and MARSHALL, R. E. Unpublished data.

[3] The ellipsod formula was applied after trials with the formula for the surface area of a sphere, viz πd, where d represents the average of three diameters of an apple, failed to give good results.

[4] WILSON. E. B. ADVANCED CALCULUS. 1912. See. p. 516.

are the same for the dotted lines as for the contour of the apple. The length of the line *aeb* is not always equal to the dotted line *afb*, and the length of the line *cgd* is not always equal to the dotted line *chd*.

Figure 2 shows what the outline of an apple looks like if it is considered as an ellipsod with diameters equal to the diameters of the McIntosh apple shown in figure 3, *C*. The surface areas of ellipsoids, similar to the one shown in figure 2 constructed from measurements of the McIntosh apple in figure 3, *C*, were found by the formula

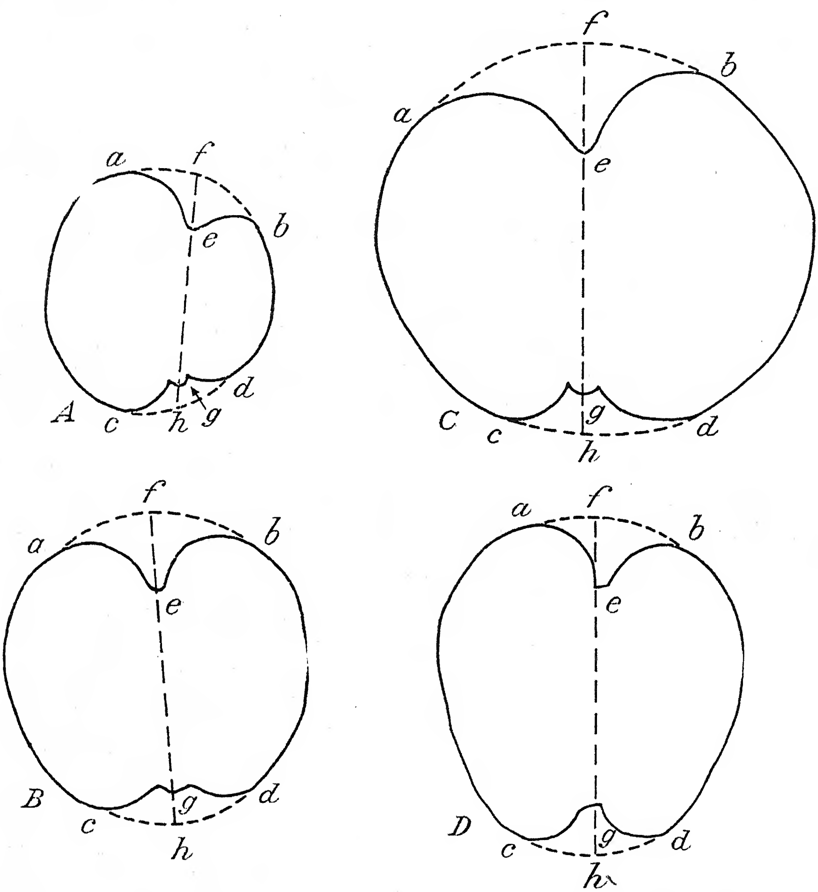


FIGURE 3.—Drawings showing axial cross sections of Jonathan apple No. 29 (*A*), Jonathan No. 28 (*B*), McIntosh (*C*), and Delicious (*D*). Dotted lines *afb* and *chd* show the approximate ellipsods that would result if the area of the skin in the stem cavity and basin were pulled out as shown.

given above. Of course an ellipsoid corresponding to each apple was not actually drawn as in figure 2. The surface area of an ellipsoid corresponding to each apple (by using dotted lines as shown above) was computed by the above formula and compared with the surface area found by the planimeter. Figure 3, *A* and *B*, shows the dotted lines for Jonathan apples Nos. 29 and 28, respectively.

The surface areas of these ellipsoids approximate closely the corresponding areas found with the planimeter.

A study of the differences in surface area obtained by the formula and those obtained by the planimeter showed that all were in absolute value less than 0.9 of a square inch and that only 6 were as large as 0.5 of a square inch. These facts, together with the plus and minus differences, show that formula 1 gives approximate values for the surface areas of Jonathan apples. The average error or the average difference is 0.03 square inch, and the standard deviation of these errors, which may be considered to be the standard error of estimate, is 0.35 square inch (table 1). The 43 positive and 30 negative differences indicate that formula 1 gives values a little too small since the error is considered to be the planimeter value minus the value obtained from formula 1. Formula 1 was applied to McIntosh, Delicious, and Stayman Winesap apples, with the results given in table 1.

Figure 3, *C* and *D*, shows how the rounding at the stem and blossom ends of McIntosh and Delicious was made by the dotted lines. An apple is not an ellipsoid (a section through the axis does not cut the apple into two equal parts), but the surface area computed from the ellipsoid described is nearly equal to that obtained from the planimeter measurements (table 1). The errors pertaining to Delicious apples are larger than those pertaining to Jonathan, McIntosh, or Stayman Winesap; those for Jonathan (first harvest) being on the average the smallest. No doubt the points or crowns and accompanying corrugations at the blossom end of the Delicious fruits gave rise to errors, as did also the broad flattened shapes of the McIntosh fruits.

TABLE 1.—*Averages of the differences in surface area, in square inches, of different varieties of apples determined by formula (1) and planimeter readings*

Variety	Harvested—	Mean difference	σ	Maximum error	Percent less than 1 square inch in error
Jonathan	Sept. 5	0.03	0.35	0.87	100
Delicious	Mature	.98	1.15	2.09	81
McIntosh:					
First sample	do	.20	.63	1.20	93
Second sample	do	−.11	.63	1.76	85
Stayman Winesap	do	.68	.98	2.08	68

Since formula (1) involves two incomplete elliptic integrals, cosines of angles and radicals, it is very difficult to use. An expert computer requires about 20 minutes to calculate the surface area of one apple with this complicated formula. It is therefore not practical to employ, although it does give very good results. Other less complex formulas were developed and compared with formula (1).

SURFACE-AREA PREDICTIONS FROM TRANSVERSE CROSS SECTION

The areas of the transverse cross sections shown in figure 1 and the surface areas obtained from the planimeter are plotted in figure 4 for Jonathan and McIntosh apples at maturity. This graph and similar ones for the other varieties suggest a linear relation between the areas of the transverse cross sections and surfaces areas. The predicting

straight lines are drawn in the chart together with the bands of "normality." The width of both bands is two times the size of the standard error of estimate. This band shows on the average the in-

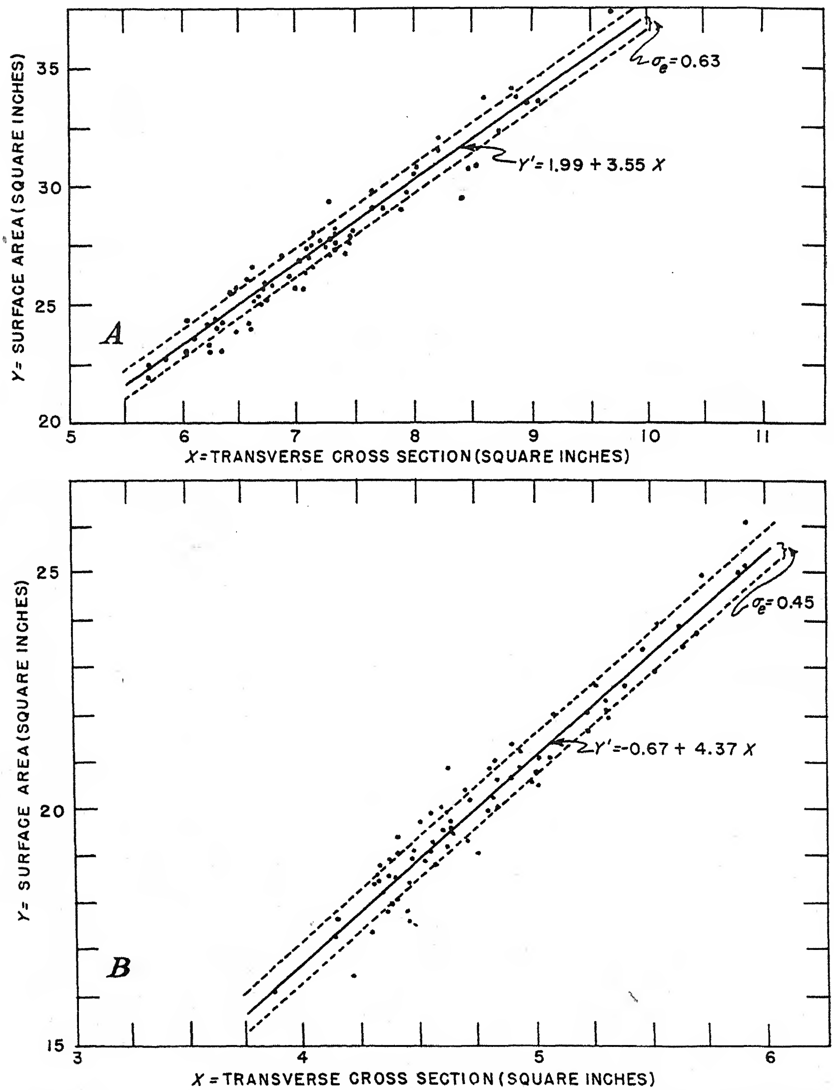


FIGURE 4.—Areas of the transverse cross sections shown in figure 1 and the surface areas obtained from planimeter readings plotted for McIntosh applies (*A*) and Jonathan apples (*B*) at maturity.

terval in which 68.2 percent of the observed values of surface areas will fall if the predicting equation is used for estimating the surface area. In this chart the scatter diagrams fall along the straight lines,

indicating a rather definite association between these two variables. The size of the standard error of estimate reveals the magnitude of the average error when the predicting equation is used. Table 2 contains the predicting equations for the varieties for the different harvesting dates, together with the standard errors of estimate. These equations were found when $y=a+bx$ and $y=cx$ were used as the linear relation between planimeter surface area and area of transverse cross section. If the apples were perfect spheres, the surface area would be equal to $y'=4x$. The constant c is in the neighborhood of 4.

TABLE 2.—*Equations for predicting surface area from the area of transverse cross section* [1] *of apples of different varieties harvested at different times*

Variety	Harvested—	Mean surface area	Equation	σ
		Square inches		*Square inches*
Jonathan	Sept. 5	13.17	$y'=-0.05+4.11x$	0.51
			$y'=4.09x$	.56
	Sept. 20	17.10	$y'=.23+4.13x$	.54
			$y'=4.19x$	.55
	Oct. 24	20.31	$y'=-.67+4.37x$	.45
			$y'=4.24x$	.46
Delicious	Sept. 14	17.24	$y'=-.34+4.07x$	.56
			$y'=3.99x$	.56
	Sept. 28	17.04	$y'=.40+3.97x$	.82
			$y'=4.06x$	.83
	Oct. 22	21.72	$y'=2.02+3.83x$	.88
			$y'=4.22x$	.92
McIntosh	Mature	27.67	$y'=.38+3.77x$	.77
			$y'=3.82x$	.78
	do	22.62	$y'=1.99+3.55x$	.63
			$y'=3.69x$	.66
Stayman Winesap	do	24.35	$y'=.84+3.92x$	1.12
			$y'=4.06x$	1.13

[1] x=area of transverse cross section; y' =predicted value.

The sizes of the standard errors of estimates show that one can predict the surface area of apples from the equation $y'=cx$ about as well as from the equation with a constant in it, $y'=a+bx$. This means that the surface area is equal to a constant factor times the area of the transverse cross section. The errors on the average in table 2 are larger than the errors in table 1, showing that formula (1) is the better, except in the case of Delicious apples.

SURFACE-AREA PREDICTIONS FROM LONGITUDINAL SECTION

When surface areas were predicted from the areas of the longitudinal or axial cross sections shown in figure 1, the results recorded in table 3 were obtained. If the standard errors of estimate in table 2 and table 3 are compared, it will be seen that those in table 3 are a little larger, except in the case of Stayman Winesap. This shows that the areas from transverse cross sections result in scatter diagrams that fit the lines for predicting surface areas a little better than the areas from longitudinal cross sections; the differences are not very large.

TABLE 3.—*Equations for predicting surface area from the area of axial or longitudinal cross section*[1] *of apples of different varieties harvested at different times*

Variety	Harvested—	Equation	σ_e
			Square inches
Jonathan	Sept. 5	$y'=0.62+4.33s$	0.62
		$y'=4.54s$	.62
	Sept. 20	$y'=.11+4.61s$	.60
		$y'=4.64s$	.60
	Oct. 24	$y'=.72+4.52s$	.68
		$y'=4.69s$	.69
Delicious	Sept. 14	$y'=.81+4.13s$	.82
		$y'=4.33s$	.82
	Sept. 28	$y'=.23+4.32s$	.78
		$y'=4.38s$	.78
	Oct. 22	$y'=2.54+4.05s$	1.01
		$y'=4.57s$	1.06
McIntosh	Mature	$y'=.05+4.84s$	1.36
		$y'=4.85s$	1.36
	do	$y'=3.66+3.88s$	.88
		$y'=4.62s$	.96
Stayman Winesap	do	$y'=2.20+4.30s$	1.01
		$y'=4.72s$	1.07

[1] s=longitudinal cross section.

SURFACE-AREA PREDICTIONS FROM DIAMETER MEASUREMENT

Since the areas of cross sections usually are not easy to calculate, equations for estimating surface areas were obtained from the largest or transverse diameters and then from the longitudinal or vertical diameters (distance from the bottom of the cavity to the bottom of the basin). These predicting equations together with their standard errors of estimate are given in tables 4 and 5.

TABLE 4.—*Equations for predicting surface area from the transverse diameter*[1] *of apples of different varieties harvested at different times*

Variety	Harvested—	Equation	σ_e
			Square inches
Jonathan	Sept. 5	$y'=-13.09+12.65t$	0.64
		$y'=6.42t$	1.21
	Sept. 20	$y'=-16.45+14.48t$	.60
		$y'=7.41t$	1.21
	Oct. 24	$y'=-18.22+15.40t$	.68
		$y'=8.14t$	1.16
Delicious	Sept. 14	$y'=-17.21+14.51t$	.63
		$y'=7.29t$	1.31
	Sept. 28	$y'=-15.70+13.94t$	.73
		$y'=7.29t$	1.39
	Oct. 22	$y'=-19.18+15.77t$	.97
		$y'=8.41t$	1.56
McIntosh	Mature	$y'=-25.94+17.39t$	.93
		$y'=9.01t$	1.95
	do	$y'=-16.86+14.24t$	.81
		$y'=8.18t$	1.20
Stayman Winesap	do	$y'=-21.43=16.38t$	1.07
		$y'=8.75t$	1.94

[1] t=transverse diameter.

TABLE 5.—*Equations for predicting surface area from axial or longitudinal diameters* [1] *of apples of different varieties harvested at different times*

Variety	Harvested—	Equation	σ_e
			Square inches
Jonathan	Sept. 5	$y' = 0.03 + 9.83l$	1.77
		$y' = 9.85l$	1.77
	Sept. 20	$y' = 2.73 + 10.20l$	1.83
		$y' = 12.13l$	1.85
	Oct. 24	$y' = 6.49 + 9.36l$	1.77
		$y' = 14.65l$	1.89
Delicious	Sept. 14	$y' = -3.58 + 12.54l$	1.71
		$y' = 7.21l$	1.74
	Sept. 28	$y' = -3.82 + 12.84l$	2.04
		$y' = 10.51l$	2.06
	Oct. 22	$y' = 8.67 + 8.15l$	2.38
		$y' = 13.69l$	2.58
McIntosh	Mature	$y' = -1.50 + 16.56l$	3.17
		$y' = 15.72l$	3.18
	do	$y' = 8.14 + 10.25l$	1.92
		$y' = 15.97l$	2.03
Stayman Winesap	do	$y' = -5.33 + 19.65l$	2.86
		$y' = 16.13l$	2.88

[1] l=longitudinal diameter.

Values of the standard errors of estimate in tables 4 and 5 show that the transverse diameter is better to use for predicting surface area than the longitudinal or axial diameter. This is as it should be since the longitudinal diameter does not reveal the bulges or ridges of angular fruits. By comparing values in tables 2, 3, and 4, it is observed that predicting from the transverse diameter in most cases gives results about as good as predicting from areas of cross sections. The standard errors of estimate are recorded in table 6 for comparison. Figure 5 shows the relation between surface areas and transverse diameter measurements. The dots in these charts fall very close to the straight lines; the bands of "normality" are rather small for each set of data and for each variety. Measurements of the transverse diameters can be made easily and quickly with calipers on uncut apples.

TABLE 6.—*Standard errors of estimate obtained by predicting surface areas from areas of cross sections and transverse diameters* [1] *of apples of different varieties harvested at different times.*

Variety	Harvested	Measurement	σ_e
			Square inch
Jonathan	Sept. 5	Transverse cross section	0.51
		Axial cross section	.62
		Transverse diameter	.64
	20	Transverse cross section	.54
		Axial cross section	.60
		Transverse diameter	.60
	Oct. 24	Transverse cross section	.45
		Axial cross section	.68
		Transverse diameter	.68
Delicious	Sept. 14	Transverse cross section	.56
		Axial cross section	.82
		Transverse diameter	.63
	28	Transverse cross section	.82
		Axial cross section	.78
		Transverse diameter	.73
	Oct. 22	Transverse cross section	.88
		Axial cross section	1.01
		Transverse diameter	.97
McIntosh	Mature	Transverse cross section	.77
		Axial cross section	1.36
		Transverse diameter	.93
	do	Transverse cross section	.63
		Axial cross section	.88
		Transverse diameter	.81
Stayman Winesap	do	Transverse cross section	1.12
		Axial cross section	1.01
		Transverse diameter	1.07

[1] $y' = a + b\ (x, s, \text{or } t)$

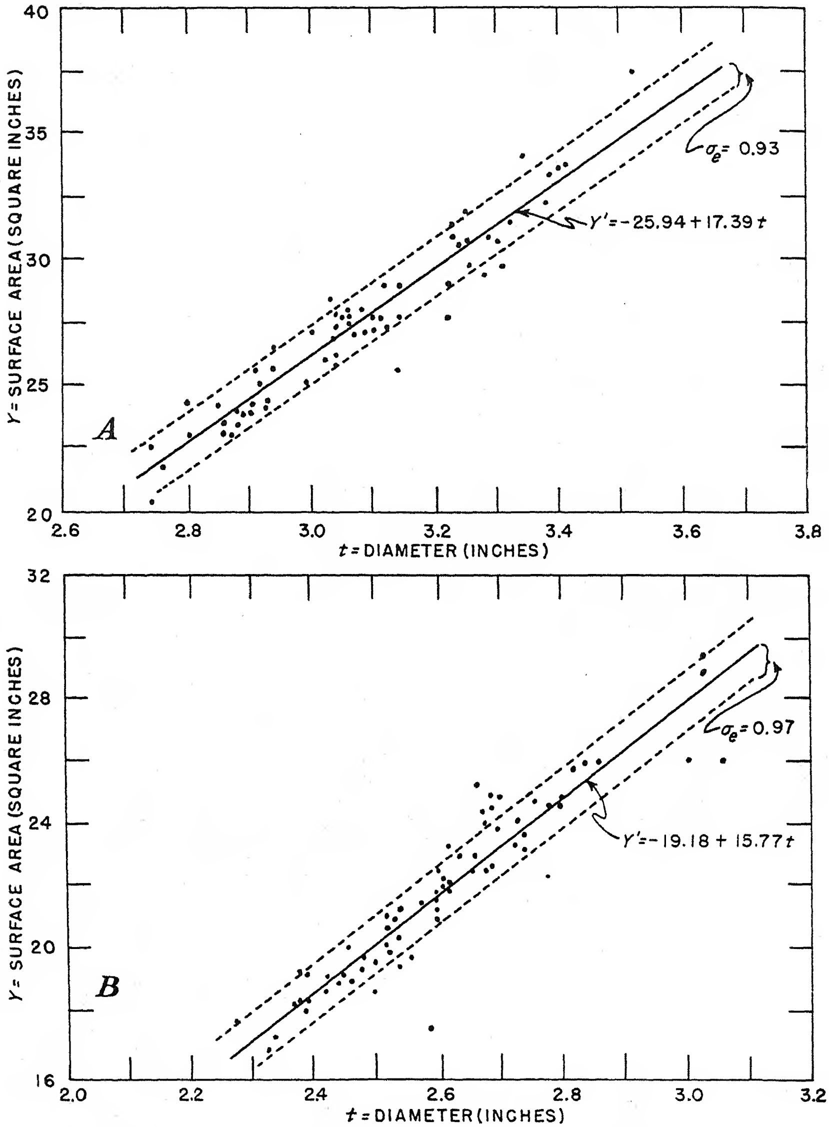


FIGURE 5.—The relation between surface area and transverse diameter measurements of McIntosh apples (*A*) and Delicious apples (*B*).

SURFACE-AREA PREDICTIONS FROM WEIGHTS

The last pickings of Jonathan and Delicious apples in 1940 were weighed, and the weights were plotted against corresponding surface areas of the fruits. A very definite linear relation between weight and surface area resulted (table 7). Since very good results were obtained from weights of the two varieties, 75 McIntosh apples and 75 Stayman Winesap apples were secured from local stores for further study.

TABLE 7.—*Equations for predicting surface areas from weight* [1] *of apples of different varieties, 1940 and 1941*

Variety and year grown	Mean surface area	Equation	σ_e	$100\frac{\sigma_e}{M_y}$ [2]	$100\frac{\sigma_e}{M_y}$ [3]	r_{yw}
	Square inches		*Square inch*	*Percent*	*Percent*	
Jonathan:						
Mature, 1940	20.31	$y'=6.437+0.136w$	0.48	2.31	3.16	0.97
Picked Sept. 11, 1941	16.94	$y'=5.194+.147w$	.58	3.42	3.79	.96
Picked Sept. 23, 1941	18.22	$y'=5.307+.146w$	.52	2.85	2.96	.96
Delicious, 1940	21.72	$y'=6.623+.129w$	.46	2.12	2.14	.99
McIntosh, 1940	22.62	$y'=7.122+.129w$	.40	1.77	2.25	.98
Stayman, Winesap, 1940	24.35	$y'=8.420+.112w$	.62	2.55	4.68	.99
Wagener, 1941	25.17	$y'=7.085+.121w$	.58	2.30	4.18	.98
Gravenstein, 1941	25.49	$y'=8.241+.116w$	.52	2.04	3.78	.96
Chenango, 1941	19.29	$y'=6.906+.128w$	.58	3.01	3.52	.94
Grimes Golden, 1941	17.25	$y'=5.889+.125w$	.70	4.06	7.64	.92
Average		$y'=6.722+.129w$				

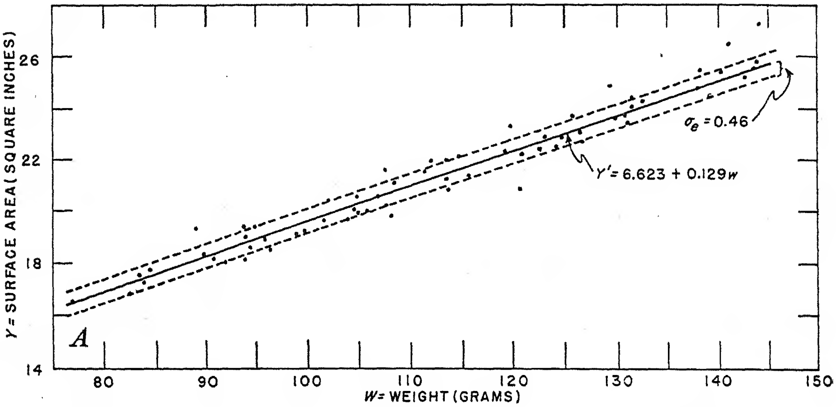
[1] w=weight.
[2] "Coefficient of variability."
[3] Obtained from average equation.

In view of the excellent results obtained for predicting surface area from weight with four varieties of the 1940 crop, it seemed advisable to enlarge this phase of the study the following year with additional varieties of various shapes. Weight and surface area measurements were therefore made during the fall of 1941 on the following varieties: Wagener, Gravenstein, Chenango, Grimes Golden, and Jonathan. In the case of Jonathan, one picking was made about 3 weeks before the commercial picking date for that variety, and a second picking was made approximately 10 days before full maturity. These, together with the Jonathans picked when mature in 1940, provided three stages of maturity for this variety. Table 7 shows the predicting equations, standard errors of estimate, "coefficients of variation," and correlation coefficients for the several varieties grown in 1940 and 1941.

The standard errors of estimate indicate that the predicting equations give splendid results for estimating surface areas from weights. The largest error is 0.70 square inch. The "coefficient of variability" $\left(100\frac{\sigma_e}{My}\right)$ is the ratio of the standard error of estimate to the mean, the ratios being expressed as percentages. In every case, these values are less than 4.1 percent; this again shows the accuracy of the predicting equations. The large values of the correlation coefficients, shown in the last column of table 7 (r_{yw}), also indicate excellent results (see figs. 6 and 7).

A study of table 7 shows that the slopes of the equations are about the same; the difference between the largest slope, 0.147, and the smallest slope, 0.112, is only 0.035. There is not a great deal of difference between the values of the constant terms in these equations. These facts suggest that one predicting equation might be used for all varieties for predicting surface area from weight. The equation, obtained by averaging the equations for the varieties listed in table 7, was found to be $y'=6.722+0.129W$.

The predicted value for each apple was determined by this equation. The deviations of the observed surface areas from predicted surface areas were then found for each fruit. The "standard errors of estimate" were obtained for each variety by finding the square root of the average of the squares of the deviations from this average equation; these were compared with the former standard errors of estimate (table 7, column 4) and also with the means. In only one case did the "coefficient of variability" exceed 5 percent or in only one case did the new standard errors of estimate exceed 5 per-



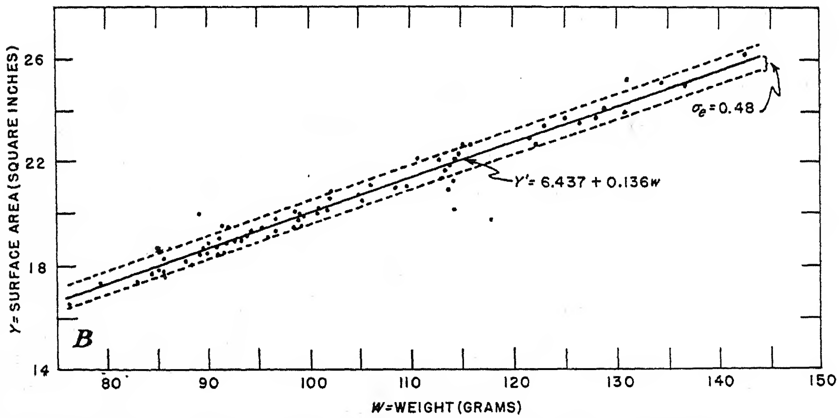


FIGURE 6.—The relation between weight and surface area of Delicious apples (*A*) and of Jonathan apples (*B*).

cent of the respective mean; this was for the variety Grimes Golden. The percentage in this case was 7.64 (table 7). These facts suggest that one relationship between weights and surface areas might be used for approximating surface areas from weights for any variety of apple.

It is interesting to note that the predicting equations for Jonathans picked on September 11 and September 23, 1941, are almost identical, which suggests that during the latter part of the growing season, the relation beween weight and surface area remains the same.

The question arises as to whether weights are better for predicting surface areas of apples than transverse sections. On examining the errors of estimate in tables 2 and 7, it is seen that for Jonathan apples one is as good as the other, but for Delicious and Stayman Winesap weight is better. A test to determine whether or not the weight is better than the transverse-section measurement for estimating surface

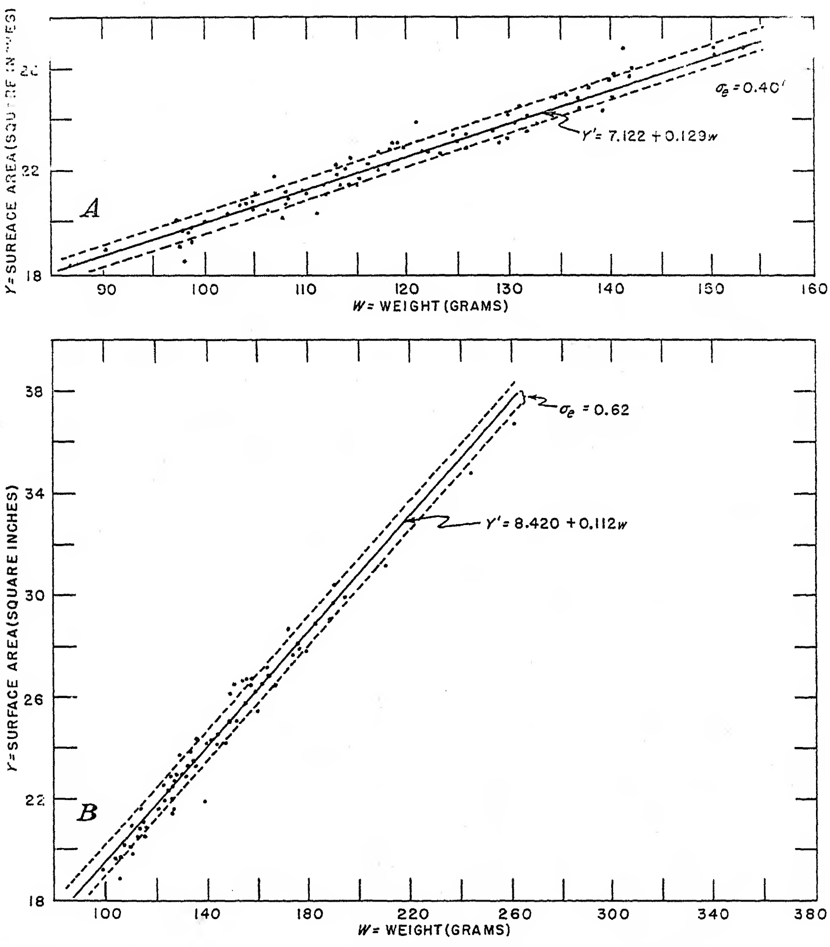


FIGURE 7.—The relation between weight and sufrace area of McIntosh apples (*A*) and of Stayman Winesap apples (*B*).

area for the McIntosh variety follows. The error of estimate when weights are used is 0.40; the error of estimate when transverse section is used 0.63. Are these significantly different? To obtain information on this point Hotelling's [5] test was employed. This test is given in detail because it is a very useful one and can be employed in many instances for determining which of two variables is better for predict-

[5] HOTELLING, H. THE SELECTION OF VARIATES FOR USE IN PREDICTION WITH SOME COMMENTS ON THE GENERAL PROBLEM OF NUISANCE PARAMETERS. Ann. Math. Statis. 11: 271-283. 1940.

ing a third variable. Applications of this test are given elsewhere.[6] The test is made by finding the following quantities:

$$t=(r_{yw}-r_{yx})\sqrt{\frac{(n-3)(1+r_{xw})}{2D}}$$

where

$$D=\begin{vmatrix} 1 & r_{yx} & r_{yw} \\ r_{yx} & 1 & r_{xw} \\ r_{yw} & r_{xw} & 1 \end{vmatrix},$$

where r_{yx}, r_{yw}, and r_{xw} are respectively the correlation coefficients between surface area y and transverse section x, between surface area and the weight w, and between transverse section and weight. In the case under consideration, the above quantities are:

$$t=(0.983-0.959)\sqrt{\frac{72(1.968)}{0.0042}}=4.408,$$

$$D=\begin{vmatrix} 1 & 0.959 & 0.983 \\ 0.959 & 1 & 0.968 \\ 0.983 & 0.968 & 1 \end{vmatrix}=0.0021.$$

On entering a t table at 72 degrees of freedom, it is found that there is a significant difference between these correlation coefficients and also the errors of estimate, or that weights are better for predicting surface areas than transverse sections of McIntosh apples.

Since weight and volume of fruits are closely related, surface-area formulas could be developed for predicting or estimating surface area from volume measurements. Such would be workable only with detached fruits, and since apples float, weight measurements would appear to be more practical than volume measurements.

RELATION OF SURFACE AREA AND WEIGHT OF PEARS

Surface area and weight measurements of Anjou, Bosc, and Bartlett pears were made in 1941 by the same methods that were employed with the apples. These varieties were used because they represent three distinct and characteristic shapes of pears. Hedrick[7] describes the shapes of these varieties as follows: Anjou, oblong-obovate-pyriform; Bosc, acute-obovate-pyriform; Bartlett, oblong-obtuse-pyriform.

Table 8 contains equations for predicting surface area from weight, standard errors of estimate, "coefficients of variability", and correlation coefficients for the pears. This table shows clearly that the surface areas for these varieties can be estimated very accurately from weights of fruits. The three predicting equations differ slightly from each other, which suggests that one equation might be used for all varieties. The equation resulting from the average of these three

[6] BATEN, W. D. HOW TO DETERMINE WHICH OF TWO VARIABLES IS BETTER FOR PREDICTING A THIRD VARIABLE. Amer. Soc. of Agron. Jour. 33: 695–699, illus. 1941.

[7] HEDRICK, U. P. CYCLOPEDIA OF HARDY FRUITS. 370 pp., illus. New York. 1922.

equations was used to estimate the surface area of each pear. This equation is $y'=7.4904+0.0995w$. Differences were found between the observed values and the predicted values from this average equation. The square root of the average of the squares of the differences was found for each variety and compared with the standard errors of estimate listed in table 8. There was very little difference between these values as is seen by examining the fourth and fifth columns in table 8. Of course, the individual equations found by the method of least squares should lead to the smaller errors of estimate. The results given above show that the equation found by taking the average of the equations can be used for estimating surface areas from weights rather accurately.

TABLE 8.—*Equations for predicting surface areas from weights of pears*

Variety	Mean surface area	Equation	σ_e	"σ_e" from average equation	$100\left(\frac{\sigma_e}{My}\right)$	r_{yw}
	Square inches		*Square inch*	*Square inch*	*Percent*	
Anjou	23.05	$y'=7.4004+0.0973w$	0.597	0.777	2.59	0.98
Bosc	22.18	$y'=7.4876+ .1008w$	.795	.801	3.58	.96
Bartlett	22.54	$y'=7.5833+ .1005w$	.693	.729	3.07	.97
Average		$y'=7.4904+ .0995w$				

RELATION OF SURFACE AREA AND WEIGHT OF PLUMS

Two fully matured varieties of plums having somewhat different shapes were studied in 1941. The Monarch variety is a round-oval plum, and the Pond is ovate to obovate in shape. Seventy-five fruits of each were weighed and the surface areas determined, the same methods being used that were employed with the apples and pears.

Table 9 shows the predicting equations, the standard errors of estimate, "coefficients of variability," and correlation coefficients. Although only two varieties were studied, it appears that the equation found by averaging the equations for the two varieties may be used with a fair degree of accuracy for determining the surface areas of varieties of plums.

DISCUSSION

Several different kinds of measurements of apple fruits grown in 1940 were made in an effort to develop an equation that might be used with a fair degree of accuracy to predict surface areas of these fruits. Each of these measurements gave straight line relationships with surface area, thus permitting use of the equation $y=a+bx$ in predicting approximate surface areas. The fruit measurement that resulted in the smallest average and the most uniform standard errors when plotted against actual surface-area measurements was found to be weight of apples.

TABLE 9.—*Equations for predicting surface areas from weights of plums*

Variety	Mean surface area	Equation	σ_e	"σ_e" from average equation	$100\left(\frac{\sigma_e}{My}\right)$	r_{yw}
	Square inches		*Square inch*	*Square inch*	*Percent*	
Pond	7.01	$y'=2.491+0.138w$	0.220	0.227	3.14	0.95
Monarch	5.43	$y'=1.876+.159w$	.147	.181	2.71	.94
Average		$y'=2.184+.149w$				

Inasmuch as weight determinations of harvested or detached apples is the most practical measurement to make of the several that were studied, it was deemed advisable to enlarge upon this phase of the study with apples in 1941 and to include some varieties of pears and plums having various shapes. In view of the fact that 15 sets of determinations with these fruits resulted in only 1 case (the Grimes Golden variety of apple) where the standard error of estimate exceeded 5 percent of the mean, the authors recommend the predicting equations involving weights of fruits where harvested, or detached fruits can be used.

Furthermore, the data indicate that it is possible to use one equation for several varieties of the same kind of fruit with at least a fair degree of accuracy. The average equation for the varieties of apples studied was $y'=6.72+0.13w$. When this was used as the predicting equation for the individual apples of 10 sets of weight measurements made in 1940 and 1941, the standard error of estimate exceeded 5 percent of the mean only in the case of the Grimes Golden variety. Similar results were obtained with average equations for pears and plums.

If it is necessary to determine approximate surface areas for apples that are attached to the tree, it is suggested that the predicting equation for surface area be based on transverse diameter measurements, which can be made readily with calipers.

Predicting equations for surface areas should be based on straight-line trends or relationships for 75 fruits that represent the ranges of shapes and sizes in approximately the proportions that actually occur on the plant or the fruits being studied. When the sample of fruits used in this study contained much fewer than 75 specimens, the standard error of estimate usually exceeded 5 percent of the mean. On the other hand, there appears to be no advantage in using a larger sample if the fruits selected are truly representative of those on the plant or in the lot to be measured.

The fruits used in this study were mature or within 3 to 4 weeks of full maturity. The predicting equations for surface areas seem to be entirely satisfactory for use within the range of growth period or degree of maturity studied, and they apparently may be used with fruits much farther from full maturity.

In view of the fact that the varieties of apples, pears, and plums used in this study afforded a wide range of sizes and shapes, the

authors believe that transverse diameter or weight measurements may be used to predict surface areas of fruits throughout the growing season, provided a representative sample of 75 fruits is used as a basis for the formulation of the predicting equation.

SUMMARY AND CONCLUSIONS

Surface areas of apples were obtained by halving the apple transversely, removing the peel in narrow strips, pinning these strips to a sheet of paper, tracing the outline of the peel strips with a sharp pencil, and measuring areas of the strips of peel with a planimeter.

The peel may be lifted from the cavity and basin of an apple to form an object similar to an ellipsoid, and a formula for calculating the surface area of an ellipsoid was found to give a standard error of estimate of 0.35 square inch when employed to predict surface areas of Jonathan apples.

Linear relations, predicting equations, and standard errors of estimate were determined for planimeter surface areas of apples and each of the following measurements: Areas of transverse cross sections, areas of axial or longitudinal cross sections, transverse diameters, axial or longitudinal diameters, and weights of fruits.

The standard errors of estimate obtained by predicting surface areas from areas of transverse cross section, transverse diameter, and weights of fruits were smaller than those for other measurements tried and any one of these three measurements may be used to predict approximate surface areas. A method is presented for determining which of two such variables is the better for predicting a third variable.

The most practical predicting equation for approximate surface areas of harvested apples is based on weights of fruits, and the most suitable one for use on unpicked apples is based on transverse diameter of apples.

An average predicting equation may be used for several varieties of the same kind of fruit with standard errors of estimate nearly as low as the standard error of estimate for an equation based on a single variety.

There appears to be a rather constant relationship between surface areas and weights of apples during the several weeks prior to maturity.

Weights of pears may be used to predict surface areas of these fruits, and an average predicting equation may be used with reasonable accuracy for several pear varieties having a wide range of shapes.

Weights of plums may be used for predicting surface areas of these fruits.

EFFECT OF FERTILIZER AND ENVIRONMENT ON THE ASCORBIC ACID CONTENT OF TURNIP GREENS [1]

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INTRODUCTION

Numerous investigations have been conducted to determine the effect of fertilizer on the ascorbic acid content of vegetables. The methods of experimentation employed have varied widely, particularly with respect to the plants studied, the cultural methods used, and the composition of the fertilizers applied. In early work ascorbic acid was determined by biological methods and in later studies by chemical methods. The lack of agreement in the results obtained in these investigations may probably be attributed, in part, to dissimilarities in the experimental procedures employed. The results indicate, in general, that the ascorbic acid content of plants tends to increase as the plant yield is increased by fertilizer application, and that nitrogen and potassium are the fertilizer constituents most effective in increasing the formation of this vitamin.

The present study represents one phase of an investigation conducted cooperatively in six States to determine the cause of variations in the composition of vegetables produced by the same cultural methods in various sections of those States. The purpose of the study, in which three of the cooperating States participated, was to determine the effect of fertilizer treatment and environmental conditions on the ascorbic acid content of turnip (*Brassica rapa* L.) greens. The experiments were conducted at Norfolk and Blacksburg, Va.; Stillwater, Okla.; and Experiment, Ga.

MATERIALS AND METHODS

Four fertilizer factors were studied. In order that not only the individual effects of the fertilizers but also the possible interrelated effects might be determined, a factorial experiment was planned.

A $2 \times 2 \times 2 \times 2$ factorial design consisting of 16 treatments with 2 replications at each location was used. The arrangement of treatments in each replication was randomized and different arrangements were used at each location. The treatments consisted of the application of nitrogen, phosphorus, potassium, and calcium in all possible combinations of high and low levels of nutrients; the check consisted of plants produced without fertilizer treatment, i. e., the zero level of application of all four nutrients. The 16 treatments were: NPKCa,

[1] Received for publication March 24, 1942. Approved for publication as paper No. 1 by the executive committee of the southern cooperative project, Variations in the Composition and Nutritive Value of Vegetables Grown in the South.

[2] The authors are indebted to B. L. Wade, senior geneticist in charge, U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., both for guidance in planning this project and for assistance in the statistical treatment of the data obtained in the experiments. The authors are also indebted to H. L. Cochran, associate horticulturist, Georgia Agricultural Experiment Station, R. C. Moore, assistant horticulturist, Virginia Agricultural Experiment Station, and H. H. Zimmerley, director, Virginia Truck Experiment Station, for their careful supervision of the production of the turnip greens used in these experiments.

NPK, NPCa, NP, NKCa, NK, NCa, N, PKCa, PK, PCa, P, KCa, K, Ca, and check.

Turnip seed of the Seven Top variety was supplied for the experiments by the United States Regional Vegetable Breeding Laboratory at Charleston, S. C. The seed was planted in rows 25 feet long and 30 inches apart. Each replicate consisted of 16 experimental rows with 2 border rows on each side of the area. The 2 replicates at each location were placed either side by side or end to end. Fertilizers were applied in a band 2 inches to the side of the row and 1 inch below the seed level. The fertilizers were applied at the following levels: 60 pounds per acre of nitrogen from ammonium sulphate, 60 pounds of P_2O_5 from superphosphate, 60 pounds of K_2O from muriate of potash, and 120 pounds of calcium from gypsum.

Records were kept of the maximum and minimum daily temperatures and the rainfall at each location.

Soil samples were taken after the ground was prepared for planting and before the fertilizers were applied. The samples were obtained in the following manner: A hole approximately 6 inches deep with a vertical side was dug with a garden trowel, and from the vertical side of the hole a thin, uniform slice of soil was taken from the surface to a depth of 6 inches. This operation was repeated 20 times in 2 diagonals across the field. The samples were thoroughly mixed and from the composite a sample was taken for analysis.

The pH values of the soils were determined by the use of glass electrodes. Rapid soil-test determinations for calcium, magnesium, nitrate nitrogen, and phosphorus were made by the perchlorate method of extraction devised by Dr. I. E. Miles [3]. Soil organic matter was determined by the method originally developed (*26*) [4] and later modified (*27*) by Schollenberger. Total exchange capacity and exchangeable calcium were determined by the methods of the Association of Official Agricultural Chemists (*1*).

When the first experiment was conducted at Norfolk, six samples of greens were analyzed from each experimental row. An examinaton of the results of this experiment by Immer's method (*12*) showed that there was need for a reduction in sampling error. This was achieved by increasing the number of samples to eight in the three experiments carried out in 1940. At Norfolk, Blacksburg, and Stillwater all rows of a single replicate and, in most cases, all rows of both replicates were sampled at the same time. At Experiment two collections of four samples were made on 2 different days after plants reached the proper size.

In view of the fact that the ascorbic acid content of plants varies thoroughout the day, samples were taken in the morning in each of the experiments, and all samples from a given location were collected at approximately the same time. At Norfolk and Blacksburg samples were taken from 8 to 8:30 a. m.; at Stillwater, from 7 to 7:30 a. m.; and at Experiment, at 7:30 and 9:30 a. m.

In sampling, large, medium, and small leaves were selected at random from average-sized plants. When the field was close to the laboratory, the samples were placed in a refrigerator within a few minutes after they were collected. At Stillwater, where the field was located some distance from the laboratory, the samples were

[3] Unpublished method.
[4] Italic numbers in parentheses refer to Literature Cited, p. 386.

placed in paper bags and packed at once with chipped ice in an insulated container in which they were conveyed to the laboratory.

In preparation for analysis, the leaves were thoroughly washed and rinsed in distilled water. Excess water was removed by patting them either with paper towels or cheesecloth. They were then placed in containers and stored in refrigerators at 2° to 4° C. until they were analyzed. All analyses were completed the same day on which the samples were collected. Duplicate determinations were made of the ascorbic acid content of samples from each row. Representative leaves of various sizes, selected for each sample, were cut or torn into strips which were well mixed. From them a 20-gm. sample was quickly weighed and placed in a glass mortar and covered with an acid mixture consisting of equal parts of 2 N H_2SO_4 and 0.25 N HPO_3.

At this point in the procedure there were some differences in the techniques employed. At Experiment, the method used for the determination of ascorbic acid was essentially that described by Bessey and King (*2*); in the other experiments, the method developed by Thornton (*29*) was used. In a comparision of the two methods, one of the investigators (M. S. Eheart), using both procedures to determine the ascorbic acid content of apples, found that the results obtained by the two methods were in close agreement. The same investigator found that the average percentage recovery of ascorbic acid from turnip greens was 96.9 percent for 25 samples.

The methods employed given briefly are as follows. At Experiment, the sample was ground under the acid mixture with 25 to 30 gm. of acid-washed sand, then transferred to 50-ml. centrifuge tubes. After centrifugation, the clear, supernatant liquid was poured off and the remaining material was again ground and centrifuged. This process was repeated a third time, the extracts were combined, and the volume was made up to 250 ml. with the acid mixture. Two 10-ml. aliquots of the well-mixed solution were titrated with a standardized solution of the dye 2, 6 dichlorophenolindophenol, which was added from a microburette. The dye solution, containing 60 mg. per 100 ml., was standardized daily against a solution of ascorbic acid containing 0.1 gm. Cebione (Merck) in 250 ml. of 3 percent HPO_3. The ascorbic acid solution was titrated against a 0.01 N iodine solution which was standardized against a 0.01 N As_2O_3 solution. One milliliter of 0.01 N iodine is equivalent to 0.88 mg. of ascorbic acid.

At the other laboratories, the procedure was as follows: The sample was finely ground under the acid mixture with 5 gm. of acid-washed sand. The mixture was transferred quantitatively to a 250-ml. volumetric flask and the volume was made up to 250 ml. with the acid mixture. Two portions of the well-mixed liquid were centrifuged for 1 minute, at 1,800 r. p. m. and a 5-ml. aliquot of the supernatant liquid from each portion was placed in a 125-ml. Erlenmeyer flask and titrated with the indophenol solution which was added from a microburette.

The indophenol solution was made up in Sörensen's solution, pH 7.0; 50 mg. of dye was used in 200 ml. of solution. At Norfolk and Blacksburg, the dye was standardized by the method used at Experiment except that the iodine solution was standardized against a stand-

ard solution of sodium thiosulfate. At Stillwater, a simplified method of standardization developed by Menaker and Guerrant (*17*) was used. In this method, an excess of potassium iodide is added to a measured volume of the indophenol solution in the presence of acid and the liberated iodine is titrated with a 0.01 N sodium thiosulfate solution, 1 ml. of which is equivalent to 0.88 mg. of pure ascorbic acid.

Analysis of variance of the data from each experiment was made in the usual manner. The effect of each fertilizer treatment was

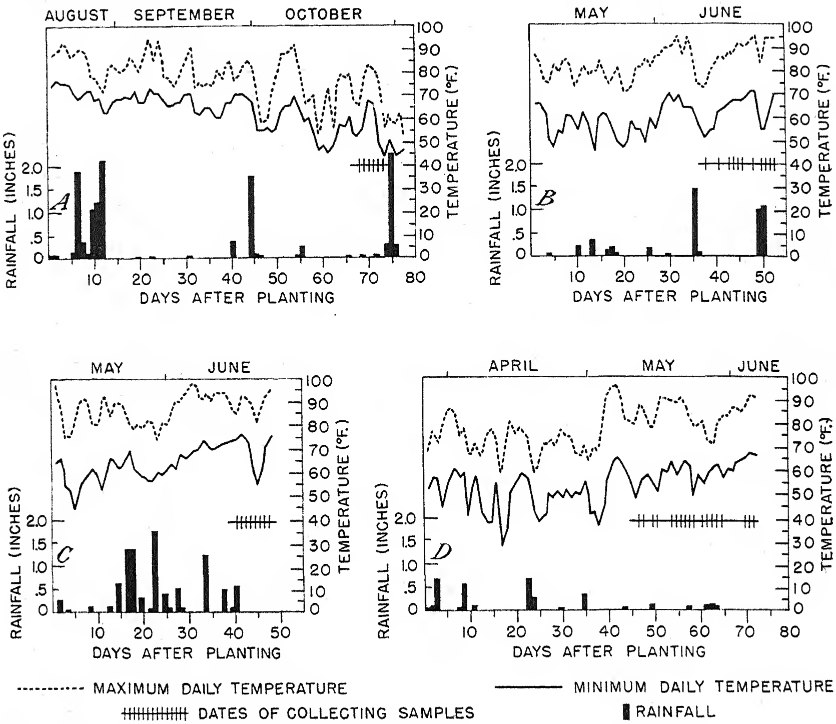

FIGURE 1.—Maximum and minimum daily temperatures, distribution of rainfall, and dates of planting and collecting turnip greens of the Seven Top variety for ascorbic acid determinations at Norfolk, Va., 1939 (*A*); Stillwater, Okla., 1940 (*B*); Blacksburg, Va., 1940 (*C*) and Experiment, Ga., 1940 (*D*).

determined by the method devised by Yates (*34*) for the analysis of factorial experiments. Chi-square tests showed the four experiments sufficiently homogeneous in error variances for satisfactory combination.

EXPERIMENTAL RESULTS

Data pertaining to the type and composition of the soil and meteorological conditions at each location during the growing season are presented in table 1. The dates of planting and collecting samples for analysis, maximum and minimum daily temperatures, and the distribution of rainfall during the growing season are shown graphically for each location in figure 1.

The average maximum temperatures at the four locations (table 1) ranged from 77.7° F. at Norfolk and Blacksburg to 83.7° at Still-

water, and the average minimum temperatures ranged from 54.2° at Blacksburg to 62.9° at Norfolk. Differences between the maximum and minimum daily temperatures (fig. 1) were smaller at Norfolk than at the other locations. The average daily rainfall (table 1) ranged from 0.06 inch at Experiment to 0.21 inch at Blacksburg. Distribution of rainfall (fig. 1) during the growing season varied considerably at the different locations. At Norfolk, 7.2 inches of rain fell during the first 13 days of the first one-third of the growing season; 2.43 inches in the second one-third; and 4.33 inches in the final period. The rains at Blacksburg were well scattered throughout the season, but the precipitation was highest during the second one-third of the period, when 6.17 inches of rain fell. At Stillwater, the rainfall was 1.26, 0.54, and 3.61 inches for the first, second, and final periods of the growing season, respectively. At Experiment, the rainfall was light and most of it fell during the first one-third of the season.

The results obtained in the 4 experiments are given in table 2, both separately and in combination. In each experiment, the average value given for the ascorbic acid content of greens receiving a given treatment is an average of the values obtained for all samples which received this treatment in the 2 replicates; in the experiment at Norfolk, this value is an average of the ascorbic acid content of 12 samples, 6 samples having been taken from a given treatment in each replicate; in the other 3 experiments, the value is an average of 16 samples.

TABLE 1.—*Type and composition of soils on which turnip greens (Seven Top) were produced, and meteorological data recorded during the growing season, at Norfolk and Blacksburg, Va.; Stillwater, Okla.; and Experiment, Ga.*

Location of experiment	Elevation	Soil type	Soil reaction	Composition of soil [1]							Meteorological data for growing period							
				Ca	Mg	N (nitrate)	P	Organic matter	Total exchange capacity [2]	Exchangeable Ca [2]	Rainfall		Average temperature		Days—			
											Total	Average daily	Maximum	Minimum	With rainfall over 0.01 inch	Clear	Partly cloudy	Cloudy
	Feet		*pH*					*Percent*	*Milligrams*	*Milligrams*	*Inches*	*Inches*	*°F.*	*°F.*	*Percent*	*Percent*	*Percent*	*Percent*
Norfolk, Va.	11	Norfolk sandy loam	5.8	L− [1]	L	M−	L+	2.47	7.6	2.3	13.08	0.17	77.7	62.9	27	32	33	35
Blacksburg, Va.	2,170	Dunmore silt loam	5.5	T+	L−	M−	L	2.42	7.2	1.6	10.2	.21	77.7	54.2	52	27	29	44
Stillwater, Okla.	880	Canadian loamy fine sand	6.0	H	M+	L+	L−	1.35	5.5	2.8	5.44	.10	83.7	59.6	25	50	29	21
Experiment, Ga.	946	Cecil sandy clay loam	5.7	L	L−	M−	L+	1.62	6.3	3.8	4.01	.06	78.8	54.4	26	49	17	34

[1] Pounds per acre for letters used in recording the results of the buffered perchloric acid test.

Elements estimated	Trace (T)	Low (L)	Medium (M)	High (H)	Very high (VH)
Calcium	0–300	−1,000	−2,000	−3,000	−4,000
Magnesium	0–10	−30	−90	−150	−200
Nitrogen (nitrate)	0–5	−20	−50	−70	−100
Phosphorus	0–10	−50	−100	−250	−400

[2] Milligrams per 100 gm. of soil.

TABLE 2.—*The ascorbic acid content of turnip greens (Seven Top) grown in factorial design of 16 duplicate fertilizer treatments at 4 locations, and the effects [1] of the treatments at each location and for the 4 locations combined*

Fertilizer treatment	Plants grown at— Norfolk, Va.		Blacksburg, Va.		Stillwater, Okla.		Experiment, Ga.		All locations	
	Ascorbic acid content [2]	Effect [3] of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content	Effect of treatment	Ascorbic acid content [4]	Effect [5] of treatment
	Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
NPKCa	2.2991	−0.0583	1.4062	−0.0093	1.4826	−0.0022	1.6194	−0.0105	1.6620	−0.0175
NPK	2.4723	−.0142	1.4013	+.0233	1.4938	−.0080	1.7769	+.0665	1.7403	+.0190
NPCa	2.3724	−.0569	1.3610	−.0126	1.5588	−.0034	1.9438	+.0634	1.7714	+.0013
NP	2.3528	−.0167	1.3934	+.0759**	1.5829	+.0017	1.8325	−.0577	1.7529	+.0020††
NKCa	2.5716	+.0268	1.2267	+.0006	1.5031	+.0166	1.7444	−.0626	1.7074	−.0067
NK	2.4281	+.0659	1.2232	+.0351	1.5468	−.0003	1.8506	−.0102	1.7178	+.0198
NCa	2.3683	−.0780*	1.2911	−.0062	1.5148	−.0257	2.0319	−.0362	1.7638	−.0337*
N	2.4873	+.0174	1.2761	+.0762**	1.5677	−.0944**	1.9950	−.1145*	1.7878	−.0292*††
PKCa	2.4129	−.0555	1.2230	+.0215	1.5982	+.0032	1.7813	−.0209	1.7099	−.0101
PK	2.2535	−.0103	1.1633	+.0193	1.5799	−.0252	1.8469	−.0535	1.6747	−.0179
PCa	2.5268	+.0124	1.2773	+.0011	1.6868	+.0187	2.0719	−.0576	1.8483	−.0076
P	2.3004	−.0730	1.2895	+.0603**	1.6239	−.0053	2.1613	−.0546	1.8133	−.0147††
KCa	2.3933	−.0094	1.1983	+.0058	1.5949	−.0111	2.0294	−.0404	1.7647	−.0141
K	2.3780	−.0183	1.2272	−.0512*	1.6316	−.0493*	1.8325	−.1927**	1.7266	−.0819**††
Ca	2.5206	+.0457	1.3061	+.0039	1.6598	−.0072	2.0594	+.0073	1.8442	+.0102
Check	2.4271		1.2842		1.6303		1.9275		1.7766	
Mean	2.4103		1.2842		1.5785		1.9065		1.7539	
Standard deviation	.2476		.1495		.1431		.3091		.2234	
Significant difference in average effects at the 5 per-cent level		.0762		.0398		.0381		.1054		.0288
Significant difference in average effects at the 1 per-cent level		.1053		.0551		.0527		.1458		.0384

F values for variance analysis at the 4 locations combined are: Places = 1,022.27**; treatments = 3.513**; treatment × places = 2.687**.
* Significant at the 1-percent level.
** Significant at the 5-percent level.
†† Interaction of treatment × places is significant at the 1-percent level.

[1] All data were used in calculating the effect of each fertilizer treatment. For example, the effect of nitrogen on the ascorbic acid content of plants grown at a given location was calculated in the following manner: After adding the replicates together to get a total for each of the 16 treatments the 8 containing nitrogen, i. e., N, NCa, NK, NKCa, NP, NPCa, NPK, and NPKCa, were added together and the 8 not containing nitrogen were added together. The difference between the positive combinations and the negative combinations is the total algebraic effect of nitrogen. (See table 3.) The effect of each of the single fertilizers at a given location was calculated in a similar manner. For the calculation of the effects of treatments containing two or more fertilizer constituents consult table 3 or Yates (*34*).
[2] Each value is an average of 16 samples from duplicate plots at all locations except at Norfolk, where the value is an average of 12 samples
[3] Each value is an average of 128 samples from 16 plots at all locations except at Norfolk, where the value is an average of 96 samples.
[4] Each value is an average of 60 samples from 8 plots.
[5] Each value is an average of 480 samples from 64 plots.

The average effect of each fertilizer treatment was calculated for each experiment and these effects are shown in table 2. The method of calculation is shown in table 3. In all cases these effects make use of more of the data than do the averages compiled from the treatment alone; for example, in each experiment only 2 rows received nitrogen, but the effect of nitrogen in the experiment is calculated on the basis of 16 rows, since half of the 32 rows in the 2 replicates received nitrogen and half did not. At Norfolk, each effect was calculated from the ascorbic acid content of 96 samples; at the other locations, from the results of the analyses of 128 samples. The significance of these results was determined and significant effects are designated in the table.

As may be seen from an inspection of table 2, five of the fertilizer treatments gave significant effects at one or more places; these were the simple fertilizers, N, P, and K and the combinations NP and NCa. The largest number of significant effects in one experiment was observed at Blacksburg, where four treatments significantly affected the ascorbic acid content of the greens. Two treatments gave significant effects at Experiment, two at Stillwater, and one at Norfolk.

Treatment with nitrogen fertilizer gave the most interesting results; at Blacksburg and Norfolk, nitrogen produced an increase in ascorbic acid content (significant at Blacksburg), but at Stillwater and Experiment, the application of nitrogen resulted in significant decreases in this vitamin. The decrease was significant for the combination of the four places, and the interaction of N treatment × places was highly significant.

TABLE 3.—*Main effects and ineractions in a 4-factor experiment*

	Combination of treatments															
	Check	Ca	K	KCa	P	PCa	PK	PKCa	N	NCa	NK	NKCa	NP	NPCa	NPK	NPKCa
Total	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ca	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
k	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+	+
kca	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+
p	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+
pca	+	−	+	−	−	+	−	+	+	−	+	−	−	+	−	+
pk	+	+	−	−	−	−	+	+	+	+	−	−	−	−	+	+
pkca	−	+	+	−	+	−	−	+	−	+	+	−	+	−	−	+
n	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
nca	+	−	+	−	+	−	+	−	−	+	−	+	−	+	−	+
nk	+	+	−	−	+	+	−	−	−	−	+	+	−	−	+	+
nkca	−	+	+	−	−	+	+	−	+	−	−	+	+	−	−	+
np	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+
npca	−	+	−	+	+	−	+	−	+	−	+	−	−	+	−	+
npk	−	−	+	+	+	+	−	−	+	+	−	−	−	−	+	+
npkaca	+	−	−	+	−	+	+	−	−	+	+	−	+	−	−	+

The effect of potassium in the fertilizer was to decrease the ascorbic acid in each of the four experiments. This decrease was significant in three places and for the combination of all places. The average decrease effected in ascorbic acid content was 8.2 mg. per 100 gm. of fresh material for 480 samples which were treated with fertilizer containing potassium, as contrasted with the same number of samples not fertilized with potassium. There was also a significant interaction between places and the treatment effect of potassium; this indicates that there was a much greater decrease due to potassium applications at Experiment than at any of the other three places.

Phosphorus gave a significant effect only at Blacksburg, where it increased the ascorbic acid content. At the other three places the effects were negative but not significantly so. The effect for P treatment × places was highly significant. Calcium was associated with an increase in ascorbic acid content in three places and with a decrease in one place, but none of the effects were significant. The interaction of Ca treatment × places was not significant.

The combination of NP gave a highly significant increase at Blacks burg, a nonsignificant increase at Stillwater, and nonsignificant decreases at the other two places. The interaction of NP treatment × places was highly significant. The combination of NK gave nonsignificant increases at two places and nonsignificant decreases at two places. The effect for the combination of four places was not significant. NCa gave decreases at four places, significant at one place, and the decrease for the combination of four places was significant.

The most striking observation to be made from the four experiments is that at Norfolk, where the maximum vitamin formation was obtained, the mean ascorbic acid content of the greens (2.4103 mg. per gm.) was nearly twice as great as that of greens grown at Blacksburg, where the mean ascorbic acid content (1.2842 mg. per gm.) was the lowest observed in the experiments. The mean ascorbic acid content of the greens produced at Experiment and Stillwater was 1.9065 and 1.5785 mg. per gm., respectively. The influence of places was 13.75 times as great as the most important average effect, i. e., the effect for potassium, but when compared to the effect of potassium at Experiment, place difference was only five times as great.

DISCUSSION

Several earlier investigators (*16*, *21*, *23*, *24*, *25*, *32*, *33*) found no changes in the ascorbic acid content of various plants as a result of fertilizer treatment. A few workers have reported that certain fertilizer ingredients altered the ascorbic acid content of some plants. None of these investigators, however, worked with turnip greens. The significant decreases in ascorbic acid produced by nitrogen in the fertilizer at two places and the significant decrease obtained by combining the results from all four places are directly opposed to the findings of a number of other workers who found nitrogen to increase the ascorbic acid content of many plants (*3*, *6*, *10*, *11*, *13*, *19*). The highly significant interaction of N treatment × places in the present experiments suggests one reason for the lack of agreement; i. e., the effect of place is more important than the effect of fertilizer.

The most consistent effect obtained in the four experiments was the decrease in the ascorbic acid content of greens which received potassium fertilizer. The opposite effect was observed by Hester (*7*), Ijdo (*10*), and others (*11*, *22*); Isgur and Fellers (*13*) and Fellers et al. (*4*) found no change in the ascorbic acid of plants as a result of potassium fertilization.

The highly significant interaction of potassium effect × places, although all places showed a decrease in ascorbic acid content, indicates that potassium reduced the ascorbic acid content more sharply in one location than in another. The greatest reduction was found at Experiment, Ga.

The effect of phosphorus in the fertilizer was not significant for the combination of the four places. This confirms the results of previous studies (*10*, *13*) with spinach and Swiss chard. Ott (*19*), however, increased the amount of this vitamin in potatoes by applying nitrogen and phosphorus, and Pfützer and Pfaff (*22*) increased it in vegetables by the addition of phosphate to a phosphorus-deficient soil. The ineffectiveness of calcium in the fertilizer in significantly modifying the ascorbic acid content of the greens in the present experiments confirms the results obtained by Ijdo (*10*) with spinach.

NCa was the only fertilizer combination to give an effect significant for the combination of the four places. Several investigators (*3*, *5*, *9*, *28*, *31*) have found that the ascorbic acid of different vegetables was increased by the application of the fertilizer combinations NPK or NPKCa, but the effects of these combinations in the present experiments were not significant. Ott (*20*) found that tomatoes grown without fertilization contained more vitamin C than those fertilized with NPK.

The results show that under the conditions imposed in these experiments, fertilizer treatment significantly affected the ascorbic acid content of turnip greens, but that the effect of place was more important than the greatest effect obtained with any fertilizer. Factors contributing to the effect of place are the type and composition of the soil and meteorological conditions during the growing season. Different types of soil were used at the four locations (table 1), but the results of the soil analyses (table 1) do not reveal very marked differences in soil composition. It is possible that trace elements in the soil exert an effect on ascorbic acid formation; Hester (*8*), for example, indicated that the amount of manganese in the soil is an important factor affecting the ascorbic acid content of plants. From the results obtained, no conclusions may be drawn as to the relationship between either soil composition or temperature and the ascorbic acid content of turnip greens.

The fact that the greens which had the highest ascorbic acid content were grown in the fall suggests a seasonal variation in the ascorbic acid content of turnip greens. No conclusion may be drawn from this fact, but the observation is made in view of the results obtained by Tressler, Mack, and King (*30*), who found that spinach produced at two locations in the fall contained one-third more ascorbic acid than spinach grown in the spring at the same locations. Because of the seasonal effect observed by these investigators, the three spring crops are compared; of these, the crop having the highest ascorbic acid content (1.9065 mg. per gm.) was grown at Experiment, where the average daily rainfall was lower than at any of the other three places, and 49 percent of the days were clear. At Blacksburg, where the greens had the lowest average ascorbic acid content (1.2842 mg. per gm.), the average daily rainfall was greatest and only 27 percent of the days of the growing season were clear. These results indicate an inverse relationship between the amount of rainfall and ascorbic acid content of the greens and a direct relationship between the amount of sunshine and the formation of ascorbic acid. Several investigators (*14*, *15*, *18*) have shown that the ascorbic acid content of plants is directly influenced by light intensity. Experiments are in progress to investigate further the effect of season and other environmental factors on the ascorbic acid content of turnip greens.

SUMMARY AND CONCLUSIONS

Experiments were conducted at Norfolk and Blacksburg, Va., Stillwater, Okla., and Experiment, Ga., to determine the effects of fertilizer treatment and environmental conditions on the ascorbic acid content of turnip greens. A factorial design was used for applications of N, P, K, and Ca in all possible combinations at a high and low level for each nutrient.

Uniform methods of planting and fertilizing were used with seed of the variety Seven Top from a single source. Meteorological data were recorded for each experiment and soil samples from each area were analyzed for calcium, magnesium, nitrate nitrogen, phosphorus, organic matter, total exchange capacity, exchangeable calcium, and pH values. Chemical methods were used for the determination of ascorbic acid. The results of the experiments were analyzed statistically, both separately and in combination, and are discussed in terms of the calculated effects of the fertilizer treatments.

Three of the single fertilizer treatments, N, P, and K, and two of the fertilizer combinations gave significant effects at one or more places. Nitrogen fertilizer gave increases in ascorbic acid at two places (significant at one place) and significant decreases at two places. The decrease for the four places combined was significant. The interaction of N treatment × places was highly significant.

The most consistent results were obtained with potassium fertilizer. which produced a decrease in ascorbic acid in each experiment; the decreases were significant at three places and highly significant for the combination of places. Many investigators working with other plants have found the application of potassium fertilizers to increase the ascorbic acid content.

Phosphorus gave a significant increase at one place and nonsignificant decreases at three places. The effect for P treatment × places was highly significant. Calcium produced no significant effects and the interaction of Ca treatment × places was not significant.

The combination of NP gave a highly significant increase at one place and the interaction of NP treatment × places was highly significant. NK gave nonsignificant increases at two places, and nonsignificant decreases at two places. The effect was not significant for the combination of places. NCa gave decreases at all places, significant at one place, and the decrease was significant for the combination of four places.

Wide variations were obtained in the ascorbic acid content of greens produced at the four places; the mean ascorbic acid content of greens at Norfolk (2.4103 mg. per gm.) was nearly twice that of greens at Blacksburg (1.2842 mg. per gm.). In the four experiments, the influence of place was 13.75 times as great as the most important average effect produced by fertilizer treatment. These variations did not appear to be directly related to differences in soil composition or to differences in temperature. Fertilizers represent at most only a small part of the total environment of a plant; consequently it could not be expected that controlled applications of fertilizer would have as much effect as the total environment involving differences in soil and weather.

Influence of season is suggested by the fact that the one fall crop had the highest ascorbic acid content, but this result is confounded

with the effect of place so that no definite conclusion may be reached. The highest ascorbic acid content of the three spring crops (1.9065 mg. per gm.) was found in greens which were produced at the place having the lowest average daily rainfall and where 49 percent of the days in the growing season were clear; the lowest ascorbic acid content (1.2842 mg. per gm.), was found in greens which received the greatest average daily rainfall and the least amount of sunshine. These results seem to indicate that the formation of ascorbic acid may be influenced by light intensity and rainfall as well as by fertilizer applications.

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THE TEMPERATURE FACTOR IN FERTILIZATION AND GROWTH OF THE BARLEY OVULE[1]

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INTRODUCTION

Temperature has long been recognized as a factor that profoundly influences growth. Minimum, optimum, and maximum temperatures have been determined in many plants for different phases of growth and have been found to differ for different species, for different organs in the same plant, and for different ages of the organ. Moreover, a definite variation occurs with the length of exposure.

As early as 1861, Hofmeister (*5*)[2] had observed that in *Crocus vernus*, under favorable conditions of warm moist air and bright sunshine during the daylight hours, the pollen tube reached the micropyle in 24 hours, whereas in cooler, drier air its passage required 48 to 72 hours. In *Trifolium pratense*, Martin (*7*) found that, during the high temperatures of July, division of the egg cell occurred 18 hours after pollination, whereas, in the much cooler October, the time required was 35 to 50 hours. Shibata (*10*) stated that he was able to lengthen the time between pollination and fertilization at will in *Monotropa uniflora* by lowering the temperature, and that almost complete suppression of fertilization occurred at 8° to 10°. He found that fertilization and early development proceeded as well at 28° as at "room temperature," with endosperm division continuing up to 30°. However, at 31° to 32° these processes were entirely suppressed.

Smith and Cochran (*11*) found that tomato pollen germinated best with pollen tube growth at its maximum for 6 to 12 hours at 85° F. (29.4° C.), after which the sample kept at 70° outstripped it. In studying the rate of pollen tube growth for the first 12 hours in *Datura*, Buchholz and Blakeslee (*3*) found that growth was 4½ times as fast at the optimum (33.3° C.) as at 11.1° C. At 37°, the highest temperature used, a slight falling off in the rate was observed.

In the cereals, the period of heading is critical. Unfavorable conditions at this time, such as hot winds, drought, or cold rainy weather, may be followed by lowered yields resulting from sterile florets or blasted spikes. The incidence of floret sterility in the Great Plains during the years 1910–12 led Salmon[3] to conclude that high temperatures alone at or slightly before heading could result in sterile florets in normal-appearing spikes. Later, he (*9*) found that temperatures of 30° to 40° C. at flowering gave high sterility in wheat.

[1] Received for publication May 18, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 402.

[3] Data on Report of Cereal Investigations at the Bellefourche Experiment Farm, Newell, S. Dak., 1912, on file with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

In rice, which is a warmer weather cereal, Kondō and Okamura (*6*) stated that 30° is optimum for the flowers.

The present study was limited to a measurement of temperature effects on a few phases of growth in a single variety of barley during the first 6 days following pollination.

MATERIAL AND METHODS

In February 1940, spikes of Manchuria barley (C. I.[4] 2330), a variety of *Hordeum vulgare* var. *pallidum* Ser. growing in the greenhouse at the Arlington Experiment Farm, Arlington, Va., were prepared, emasculated, and bagged. They were allowed to remain on the plant 4 days at an average temperature of about 14° C.; at the end of this time the flowers were well opened, with stigmas spread wide. Their culms were cut off just below the third node from the spike. These culms, each having two foliage leaves and the basal half of the boot leaf, were distributed among five Erlenmeyer flasks with their cut ends in distilled water. One flask was placed in each of five chambers carried at 10°, 15°, 20°, 25°, and 30°, respectively, and allowed to approach temperature equilibrium for 50 to 60 minutes. The spikes were then inserted into a paper cone and pollinated by dusting with heads of the same variety of barley bearing dehiscing anthers; they were then allowed to continue their growth, as earlier investigations (*8*) had shown that, in the barley embryo, the rate of growth for the first 11 days after pollination was practically the same in culms excised and placed in distilled water as in those left on the plant, when the two lots were kept side by side on the greenhouse bench. Pollinations were spaced 2 minutes apart so as to allow time to take the sample and move to the next chamber. Sampling was begun 20 minutes after pollination and continued at 10-minute intervals up to 90 minutes, and thereafter at intervals of 4, 5, and 6 hours and 1, 2, 3, 4, 5, and 6 days after pollination. All samples, consisting for the most part of five ovules, were in the killing fluid within about 1 minute of the interval indicated except on the fifth day, when the sampling was about 6 minutes late.

Good control of temperature was obtained in all chambers except that for 30° C., a small tank open at the top, which varied between 28° and 31° at sampling time. The chambers were dark except where light bulbs for heating were placed behind baffle boards. Relative humidity was not recorded. The ovules seemed to develop normally in all the chambers. In general, basal and tip ovules were rejected but every spike in a chamber was represented in a single sample. Formalin acetic alcohol was used as a killing and fixing fluid because of its relatively low surface tension and rapid penetration. Fixation was fair, but not up to the standard desired for cytological work, as in many samples the contents of the embryo sac were in abnormal positions.

The ovules were embedded in paraffin, cut sagittally 15μ thick, and stained, usually with Heidenhain's iron-alum haematoxylin. The process was greatly shortened in many of the older samples by mordanting briefly in iron alum and staining with 1/80 percent haematoxylin until the cell walls were blue.

[4] C. I. refers to accession number of Division of Cereal Crops and Diseases.

A study of the 1940 results showed that additional data were desirable, so the experiment was repeated in 1941 for the temperatures 10° and 30° C., and samples run at 5°, 35°, 40°, and 45° were added. Extra samplings were made at ages shown or suspected to be critical. The 5°, 10°, and 30° chambers, located in constant-temperature rooms in the basement of the greenhouse, held their temperatures satisfactorily, but those at 35°, 40°, and 45°, which were small tanks located in the greenhouse under glass and heated by light bulbs, varied more widely, the 35° and 40° tanks running 1° to 2° high. Excessively dry atmospheric conditions were guarded against by placing open pans of water in the 30°, 35°, 40°, and 45° chambers.

Much better fixation was obtained in 1941 by pre-fixing in acetic alcohol and completing the process in Craf. Both years' work will be considered together. Observations are based on about 1,000 ovules sectioned and stained.

EXPERIMENTAL RESULTS

Flower closing after pollination was faster with increased temperature. In the first year's experiments at 25° and 30° C., closure was complete in 1 day; in those at 15° and 20° the flowers were closed on the second day; and in those at 10°, on the fourth. After 6 days in the dark, the green color of the foliage had faded perceptibly at the higher temperatures. Alcoholic extracts of leaf tissue from homologous selected culms at the five temperatures used in 1940 showed the 10° samples to be the greenest. Using a Duboscq-type colorimeter and the 10° value as 100, the 15° sample had faded to 89.9, the 20° sample to 82.2, the 25° sample to 74.3, and the 30° sample to 72.9.

GROWTH OF POLLEN TUBE

Figure 1 shows the shortest time after pollination at which male nuclei were seen in the egg sac. At 5° the pollen tube grew very slowly, taking 140 minutes to reach the embryo sac, and the male nuclei had not made contact with the female nuclei at the end of 180 minutes. The rate of growth increased rapidly with temperature up to a maximum at 30° and 35°, when but 20 minutes were required to reach the embryo sac. At 25° the earliest occurrence of male nuclei in the egg sac was seen in the 30-minute sample, but they were then in contact with the egg and polar nuclei. At 40° no male nuclei were visible in any of the 10 ovules sampled at 20 minutes, but out of 6 ovules sampled at 30 minutes, 1 showed their presence. At this temperature, the egg and polar nuclei appeared abnormal in the first sample taken (10 minutes after pollination, or about 70 minutes' exposure to 40°). Later samples showed progressive degeneration. The effect of heat on the 45° samples was still more marked, and no evidence was found of male nuclei reaching the embryo sac at that temperature.

INCREASE IN NUMBER OF NUCLEI IN EMBRYO AND ENDOSPERM

The fertilization process, as observed in the barley ovules, is in general as follows. The two male nuclei reach their objectives at very nearly the same time. The group of three nuclei (1 ♂ and 2 ♀ polar nuclei) that is soon to produce the fusion nucleus then

migrates rather rapidly along strands of protoplasm until it takes a position at the proximal end of the mass of antipodal cells near the center of the embryo sac. Fusion and division into two daughter endosperm nuclei without cell walls then occur. These migrate along

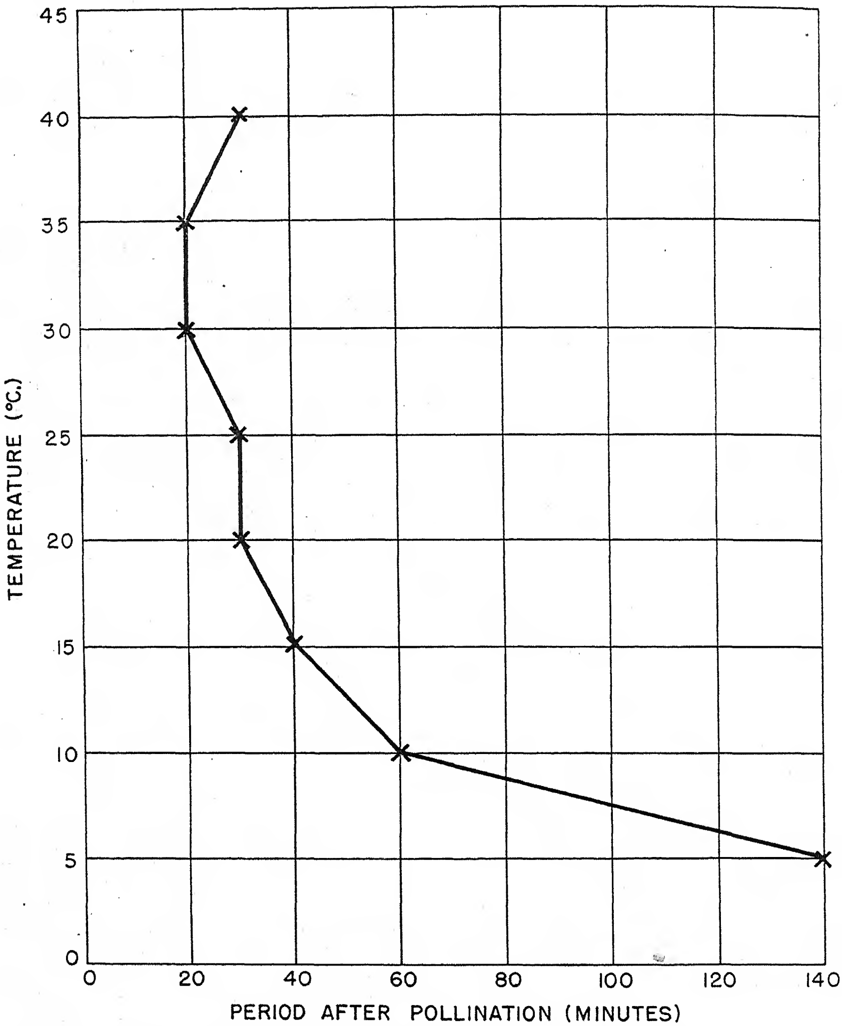

FIGURE 1.—Time from pollination required for male nuclei to enter the embryo sac of Manchuria barley when grown at constant temperatures.

the protoplasm strand and divide again. As ovule growth proceeds these protoplasmic strands gradually become a sheet of protoplasm lining the embryo sac cavity and affording free living room for a number of generations of dividing nuclei. For several generations the endosperm nuclei divide almost simultaneously and increase in number exponentially or according to the compound-interest formula

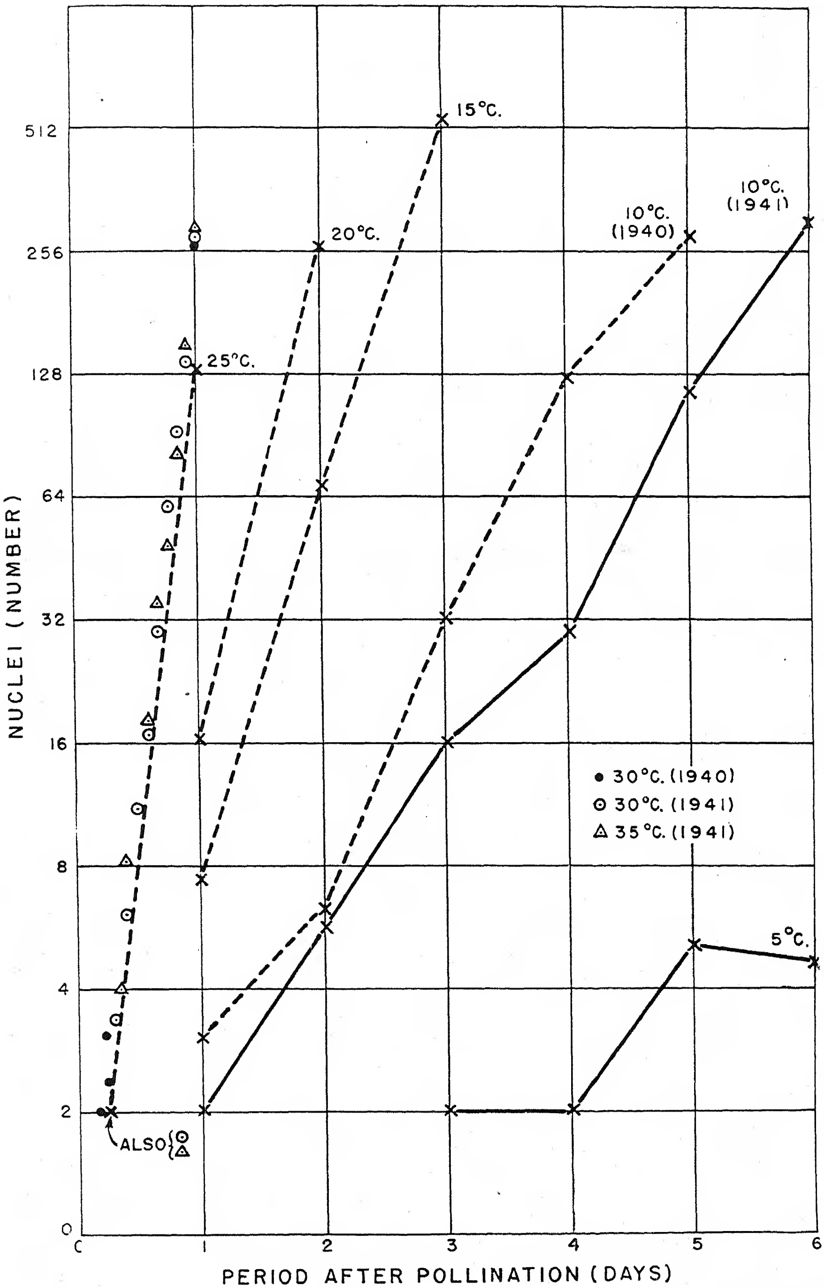

FIGURE 2.—Increase in number of endosperm nuclei in ovules of Manchuria barley growing at constant temperatures.

at 100 percent. The fertilized egg exhibits polarity from the first, division being much more frequent in the distal half. Unlike the developing endosperm, the embryo cells have cell walls. The rate of division in the young embryo is also according to the compound-

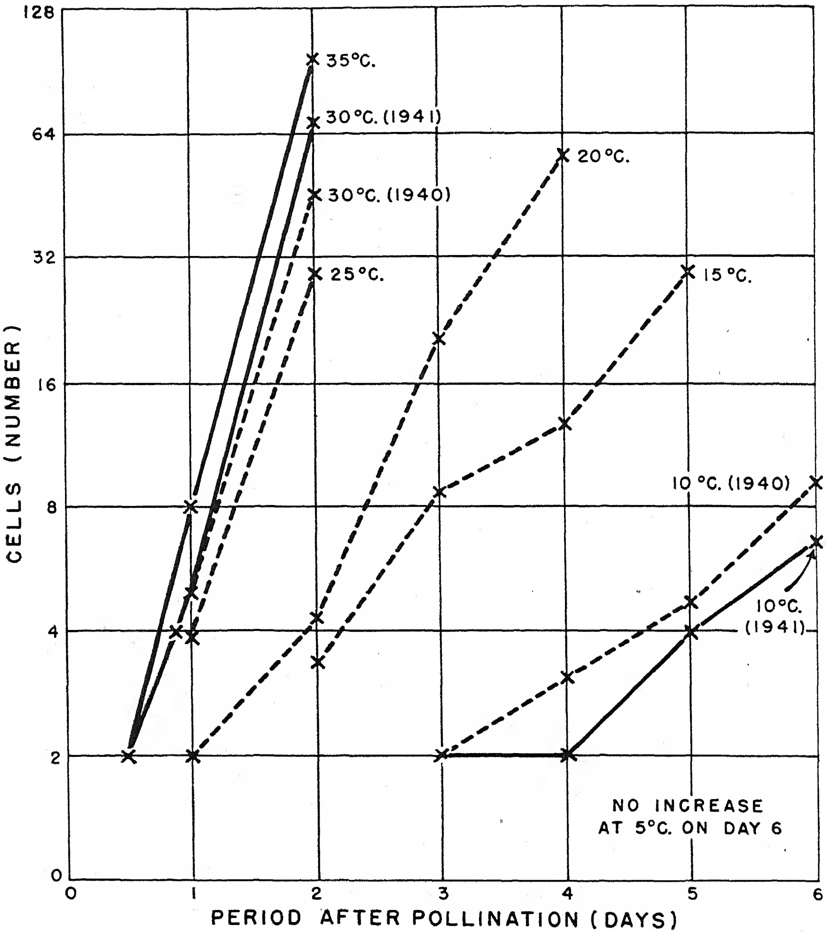

FIGURE 3.—Increase in number of embryo cells in ovules of Manchuria barley growing at constant temperatures.

interest formula, but because of its polarity, not at the 100-percent rate.

Endosperm and embryo nuclei at successive ages were counted under the microscope, but, since mitosis in the nucleus is a rapid process between two relatively long rest periods, the number of nuclei at any time may be double or but half the number that would have appeared had the sample been taken an hour or so later or earlier. The fact that nuclei were often divided by the microtome knife made exactness of counting almost impossible in the later stages, but it is believed that the numbers shown are consistent and sufficiently accurate.

The counts have been plotted with successive powers of 2 used as ordinates, which would be the number of generations where increase in number of nuclei equals compound interest at 100 percent. In the endosperm, as shown in figure 2, nucleus increase was very slow at 5° C., reaching a maximum of less than 8 nuclei, or 3 generations, in 6 days. At 10°, 9 generations occurred in 6 days in 1941, and in 5 days in 1940. At 15°, there were 10 generations in 3 days; at 20°, 8 generations in 2 days; and at 25°, 7 generations in 1 day. The increases at 30° C. for both years, and for 35° are practically

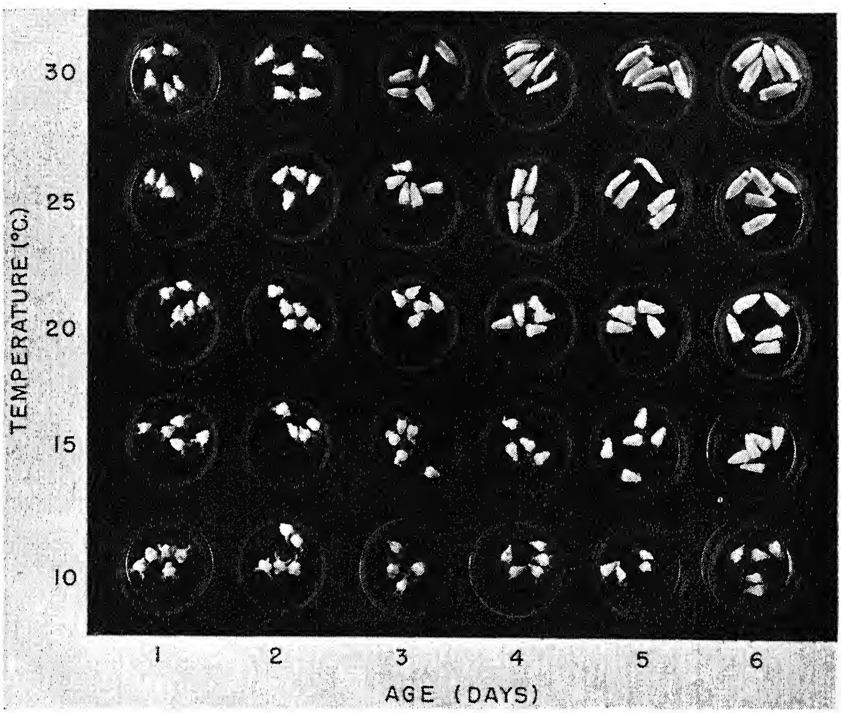

FIGURE 4.—Ovules of Manchuria barley grown at constant temperatures and harvested at daily intervals.

identical, or 8 generations at 1 day after pollination. No evidence of endosperm division was found in the 40° and 45° samples.

The curves of embryo cell increase (fig. 3) show a much slower growth than was shown in the case of the endosperm. There was no division at 5° C. in 6 days, but rate of growth increased rapidly with temperature until at 35° there were 7 generations in 2 days. At 40°, 2 young embyros were found, a 2-celled one at 1 day, which was dying, and 1 of about 46 cells, after 4 days' growth, which appeared to be dead. No embryos were found at 45°.

By way of comparison, germination tests were made of the Manchuria seed in Petri dishes in constant-temperature chambers. The sample kept at 15° C. germinated perfectly; 42 percent of the seed at 35° germinated weakly; and none germinated at 40°.

INCREASE IN LENGTH OF CARYOPSIS AND EMBRYO

The effect of temperature on kernel growth was very marked for 6 days after pollination. A photograph made of the 1940 samples is

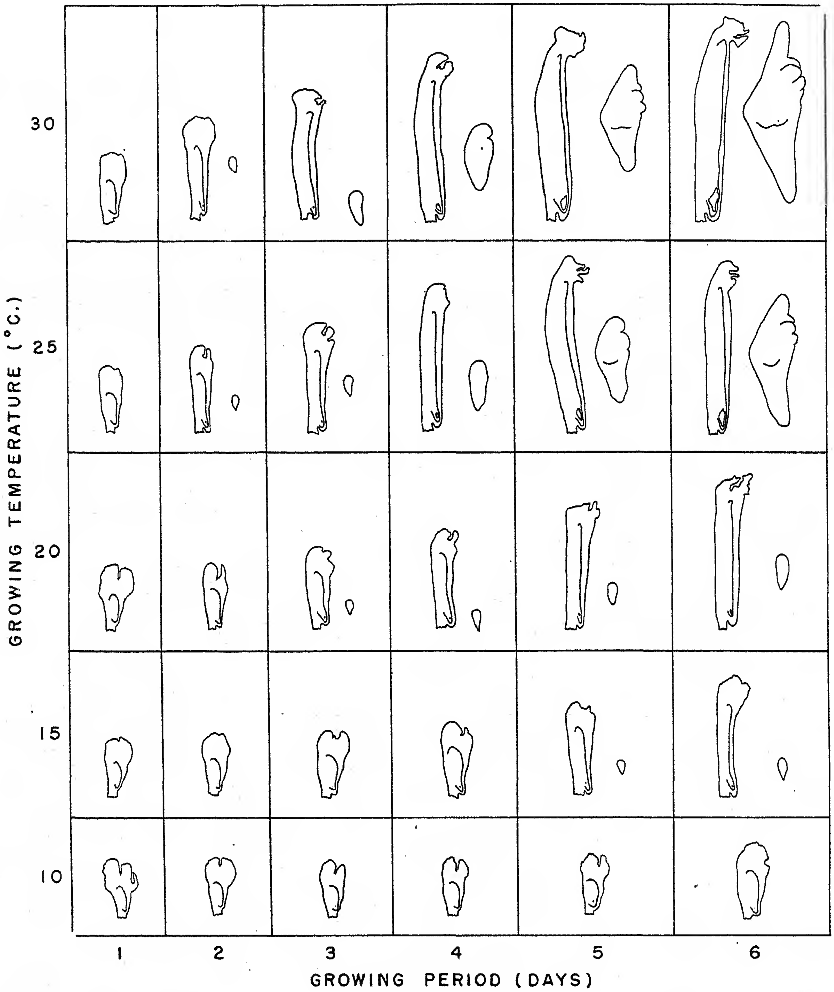

FIGURE 5.—Sagittal sections of typical ovules of Manchuria barley and their embryos grown at constant temperatures and harvested at daily intervals. Ovules, ×3.9; embryos, ×29.

shown in figure 4. The average length of the ovules grown at 30° C. for 6 days was approximately 7.02 mm.

The ovules shown there were sectioned sagittally and stained, and the median section of each was drawn with the aid of the camera lucida. Where the number of cells in the embryo exceeded 20, it too was drawn at a greater magnification. The lengths of these were determined and the ovules with their embryos most nearly approaching the average for each sample were taken as typical, and from them a chart was

constructed, as shown in figure 5, which should be useful in obtaining samples of definite stages.

After the lengths of the caryopsis and embryo were obtained from the 1941 data, the averages of the samples were plotted for all the temperatures used. The curve for caryopsis length (fig. 6) shows

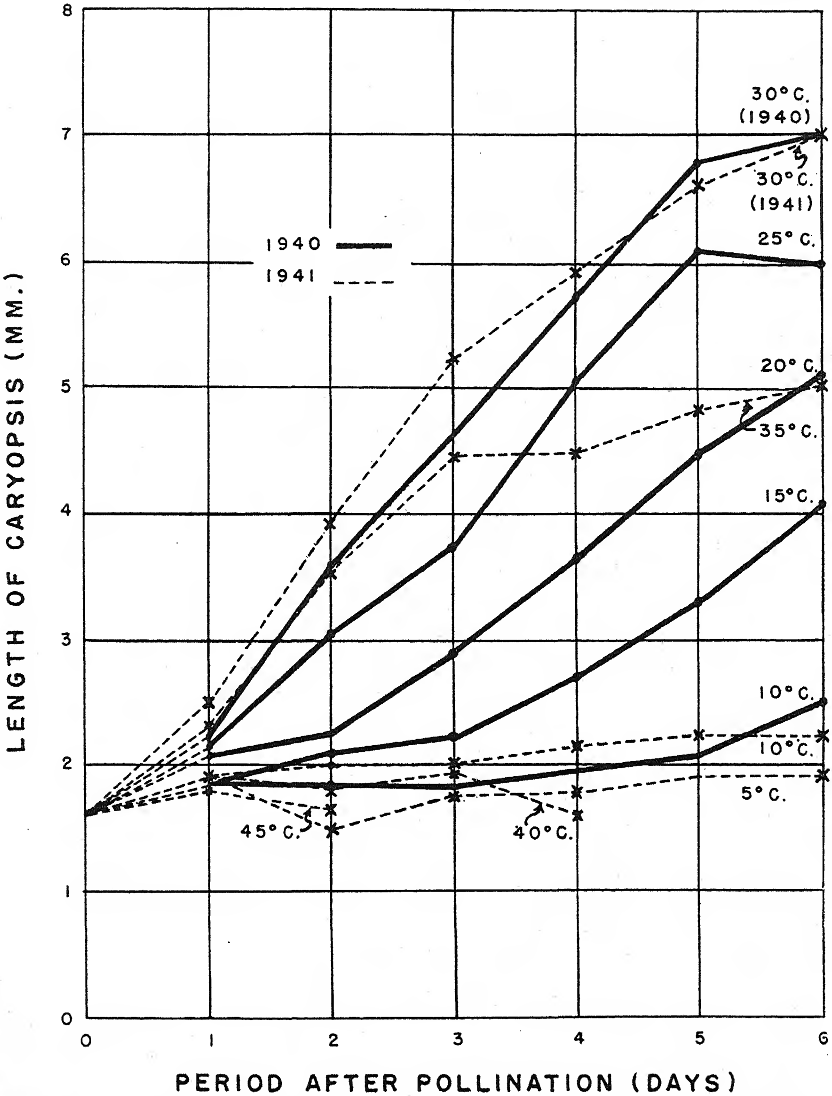

FIGURE 6.—Increase in length of caryopsis in Manchuria barley growing at constant temperatures.

very slight growth during 6 days at 5° and 10° C., but rapidly increasing growth with successively higher temperatures to a maximum at 30°. Growth at 35° was about equal to that at 30° for 3 days, but dropped rapidly thereafter. Little or no growth occurred at 40° and 45°.

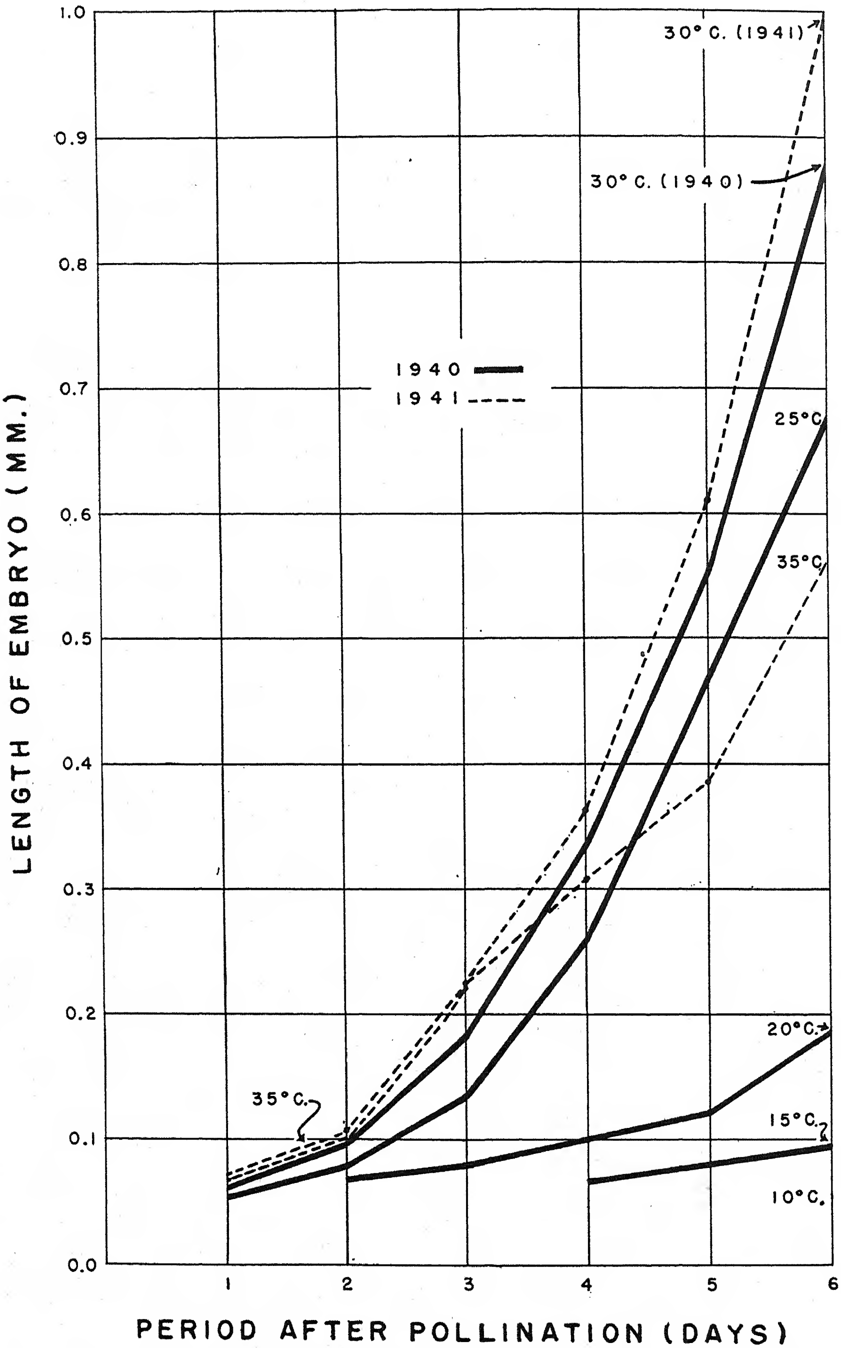

FIGURE 7.—Increase in length of embryo in Manchuria barley growing at constant temperatures.

In the embryo, the first division had not taken place after 6 days at 5° C. (fig. 7). At 10° and 15°, growth was very slight. As in the caryopsis, the maximum length was attained at 30° and growth at 35° fell off after 3 days. No curves are shown for embryo

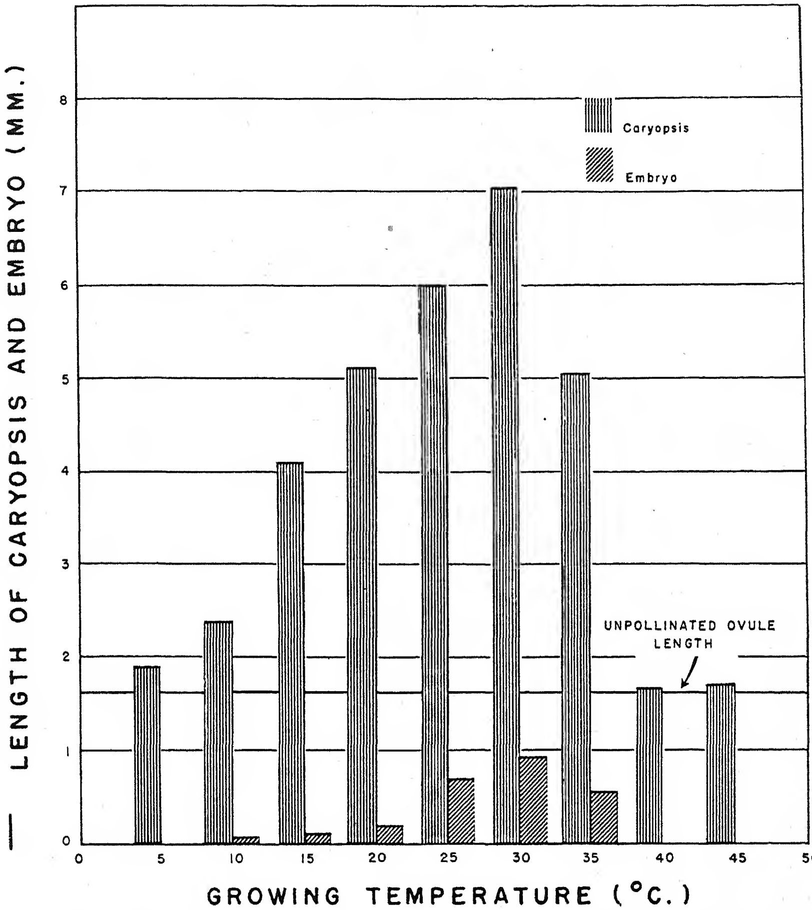

Fig. 8.—Length attained in 6 days at different growing temperatures by the caryopsis and the embryo of Manchuria barley.

growth at the two highest temperatures since fertilization was exceptional at 40° and absent at 45°.

Figure 8 compares the finally attained lengths of caryopsis and embryo at the various temperatures.

Van't Hoff's rule that velocity of a chemical reaction in homogeneous systems is doubled or trebled with an increase in temperature of 10°, C. has been found to apply to many vital processes at temperatures below the thermal death point. In table 1 are given the temperature coefficients calculated for the 10° intervals available, on the basis of increase with temperature in 6 days in number of generations of endosperm and of embryo nuclei, and in growth in length of ovule and embryo.

It is seen that the endosperm divided 5.5 times as fast at 15° C. as at 5°, but both endosperm and embryo divisions showed good agreement with the rule in the next three intervals, while at 25° to 35° the rate fell off greatly. On the other hand, growth in length of ovule and embryo showed only one close approximation; i. e., for the interval 15° to 25° in the ovule.

TABLE 1.—*Van't Hoff's temperature coefficient (Q10) for growth in Manchuria barley*

Temperature interval (°C.)	Number of Generations		Length	
	Endosperm	Embryo	Ovule	Embryo
5–15	5.5		8.3	
10–20	2.4	2.5	4.7	3.6
15–25	2.1	2.5	1.8	7.1
20–30	2.1	2.2	1.5	5.0
25–35	1.3	1.4	.8	.6

DISCUSSION

The minimum temperature for growth in the pollen tube and ovule of barley was apparently not reached at 5° C. At this temperature, fertilization progressed normally, but with no divisions in the embryo at the end of 6 days. In the endosperm, two to three divisions had taken place and the ovule had made a slight growth in length. With increasingly higher temperatures, the growth rate was markedly accelerated until at 30° and 35° the pollen tubes had completed their growth and extruded the male nuclei into the egg sac within 20 minutes after pollination. At both of these temperatures, growth of the ovule and embryo was at its maximum for the first 3 days. Since at this point the curves of the 35° samples suffered inflection, we may conclude that 30° is about the optimum temperature for these organs for a 6-day period, while 35° is close to the maximum. A similar high rate suffering early inflection was observed by Famin (*4*) in the growth of roots and shoots of *Pisum* where an initial maximum rate found at 29° later fell off and was exceeded by that of seedlings growing at 22°. Higher temperatures gave rates following much the same pattern until at 35° to 36° there was little or no growth. In the experiments of Kondō and Okamura (*6*) with rice, tillering was best at 34.5° and length growth at 30° to 32°. The rate fell off progressively at 36°, 37.5°, and 39°, and temperatures of 43.5° and 48° were lethal.

In the studies of barley reported herein, the thermal death point was reached at 40° C. Although the male nuclei were seen in one embyro sac killed 30 minutes after pollination and two very young embryos were present in later samples, no endosperm nuclei were found and no ovule appeared to be living after an estimated maximum development of 20 hours. After the spikes were given an hour's conditioning exposure before pollination, six of the seven ovules in the 40° sample taken 10 minutes after pollination showed abnormalities in the egg and polar nuclei. Progressive degeneration was evident in the egg sac of later samples. Although the flowers had been subjected to the mutilation ordinarily practiced in emasculation, and the unnaturally open interiors were exposed to the experimental temperature

for a longer time than the pollen, the evidence suggests that sterility at high temperatures may be due as much to ovule injury as to pollen killing.

From the counts of endosperm and embryo nuclei at each temperature during the first 6 days after pollination, it was found that there are approximately three divisions of the endosperm to one of the embryo. This striking difference in growth rate can hardly be explained on the basis of nutritional opportunity, since even under initially almost identical conditions the first division of the fertilized egg does not occur until there have been about three divisions in the endosperm, and during the whole time that the nuclei are countable the same ratio of division persists. Theoretically, all eight of the nuclei in the embryo sac are identical as are the two sperm nuclei; hence, in a plant homozygous from a genetic standpoint, such as the normally self-pollinated barley, the two reproductive systems present in the sac differ only in the number of reduced sets of chromosomes. Brink and Cooper (*2*) found that in alfalfa the endosperm nuclei of cross-fertilized seeds increased significantly faster than those of self-fertilized seeds, while in the embryo there was little difference in the rate of growth following self- and cross-pollination. It is suggested that the normally more rapid growth of the endosperm is due to the stimulus derived from an extra chromosome set rather than from one different in character.

Bělehrádek (*1*) stated that Van't Hoff's law may be applicable to a biological process when the temperatures are not too close or too far from the optimum and also that systematic variations of Q10 with temperature are a constant and general feature, the value of the coefficient generally increasing rapidly at low temperatures. This increase is illustrated in the Q10 value for endosperm growth between 5° and 15° C., as shown in table 1.

SUMMARY

The following observations were made on hand-pollinated Manchuria barley (*Hordeum vulgare* var. *pallidum* Ser.) grown at constant temperatures for 6 days in the greenhouse.

The most rapid pollen tube growth occurred at 30° and 35° C. where the male nuclei were present in the egg sac 20 minutes after pollination. At 5°, 140 minutes were required to reach this stage.

In countable stages, nuclear divisions occurred about 3 times as often in the endosperm as in the embryo. It is suggested that an extra set of genes in the fusion nucleus may be a major factor in the rapid growth of the endosperm.

The endosperm nuclei divided 2 or 3 times in 6 days at 5° C., and 8 times in 1 day at 30° and 35°. In the embryo there was no divison in 6 days at 5°, and 7 divisions in 2 days at 30° and 35°.

At 40° and 45° C., no endosperm divisions were found.

Two young but dying or dead embryos were found at 40° C. No embryo divisions were found at 45°.

Indications of injury to the egg and polar nuclei were present after exposure of 1 hour at 40° C.

Blasting attributed to high temperatures may be due as much to ovule injury as to pollen killing.

Optimal growth of both endosperm and embryo lies in the neighborhood of 30° C. (86° F.). Growth is less rapid at 35°., and death occurs at 40°.

Between 10° and 30° C., growth as measured by number of generations in endosperm and embryo conformed to Van't Hoff's rule on the relation of velocity of reaction to temperature.

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MECHANICAL DETERMINATION OF THE JUICINESS OF MEAT[1]

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INTRODUCTION

Flavor, juiciness, and tenderness are the most important of the factors that affect the palatability of meat. Adequate mechanical methods are available for determining tenderness, but the customary means of testing for flavor and juiciness is to submit samples of the meat to a group of judges for their opinion. The judges taste the small portions of meat and individually grade them in accordance with their own standards. Usually a chart is used as a guide to judgment and for recording opinions. The average of the opinions of the persons constituting the group has generally been accepted as the best measure of the factor under consideration.

It has long been recognized that this method has serious limitations. Individuals differ in their standards for evaluating the palatability factors and, in fact, the standard of the individual varies from time to time. Experience shows that few judges are able to discriminate well within narrow limits and that there is often a tendency to avoid extremes and to follow, more or less subconsciously, what seems to be a safe policy, that of giving intermediate grades or scores. Perhaps one of the greatest obstacles to accurate grading of any one factor of palatability is the influence of the other factors. For example, it is believed that a high degree of flavor and tenderness in a sample of meat would influence many judges to assign a higher grade for juiciness than would otherwise be the case. Possibly also the saliva of the judge would influence his decision, for its flow is known to be variable, depending on other factors of palatability, especially flavor, and on the physical condition and mental attitude of the judge. It seems logical to conclude that the diluting action of the saliva would influence the opinion of the judge with respect to juiciness.

In recent years there has been an increasing realization of the need for mechanical or chemical methods for measuring the palatability factors of meat, to replace, or at least to supplement, tests based on the opinions of judges. The principal developments in technique for measuring palatability factors have been those relating to tenderness. The juiciness factor is more complex, and efforts to develop a labora-

[1] Received for publication August 17, 1942. This study was conducted as part of the National Project Cooperative Meat Investigations. The early stages of the research leading to the development of this method were conducted as part of a cooperative project with the Bureau of Home Economics of the U. S. Department of Agriculture.
[2] The authors acknowledge their indebtedness to Edna V. Steely, of the Bureau, for statistical assistance.

tory technique for measuring this characteristic have not progressed so rapidly.

REVIEW OF LITERATURE

Several methods of determining the juice content of meat have been proposed. All involve the principle of pressure but differ in the design of the apparatus and in the mode of sampling. The early work relating to expressible juice was done on raw meat exclusively and involved no quantitative considerations with respect to comparisons of different samples. In 1905, Grindley and Emmett (*8*)[3] reported that they had obtained about 34 percent of the juice from raw beef by grinding and then pressing the meat in a compound screw press. In 1908, Bigelow and Cook (*1*) cut small pieces of beef round and chuck and pressed them through cotton bags in a glycerin cylinder press. In 1912, Botazzi, as reported by Child and Fogarty (*4*), obtained 40 and 63 percent of fluid from smooth and striated muscle, respectively, by first grinding the raw ox muscle with diatomaceous powder and then applying pressure of 350 atmospheres.

As far as the authors are aware, the first mechanical method developed for the study of juiciness of cooked meat was reported in 1934 by Child and Baldelli (*2*) of the Minnesota Agricultural Experiment Station. By means of an apparatus, called a pressometer, they subjected 2- to 3-gm. samples of meat, wrapped in unsized filter cloth, to a pressure of 250 pounds. The loss in weight of the samples they regarded as indicative of the quantity of juice originally present. The juice absorbed in the cloth was used for chemical analysis. Employing this method over a period of time, Child and her coworkers conducted studies on the effects of temperature of cooking (*2*), freezing (*6, 10*), type of beef roast (standing rib as compared with rolled rib roast) (*3*), and location of sample (*5*), on the expressible juice content of the meat. Modification of the method involving the use of different pressures in the pressometer was also studied (*2*).

In addition to the pressometer employed at the Minnesota station, a well-known make of hydraulic laboratory press has been used in several institutions. With this apparatus Hall and others at the Kansas Agricultural Experiment Station obtained juice from raw as well as cooked meat by stratifying alternate layers of ground meat and filter paper in the test cylinder.[4] From 18 to 25 percent of juice was expressed from cooked beef, but no significant correlation was found between the proportions of juice pressed from the raw and cooked lean tissues or between either of them and the judgments of a palatability committee as to the juiciness of the samples.

Noble and coworkers (*9*) applied 3,800 pounds of pressure per square inch to pieces of cooked longissimus dorsi and to a composite of certain muscles of the round of beef, which had previously been used for penetrometer readings, and obtained 15 percent of juice. With their method they studied not only different cuts but also samples cooked to different internal temperatures.

Utilizing the hydraulic laboratory press just referred to, one of the present authors[5] conducted a large number of experiments (*11*,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 412.

[4] Mimeographed report of the Kansas Agricultural Experiment Station to the Conference on Cooperative Meat Investigations, representing the United States Department of Agriculture and State agricultural experiment stations, 1934, p. 12.

[5] Nancy Griswold Clark, with the technical assistance of Virginia Weatherby, of the Bureau of Home Economics.

pp. 9–10), some of which are unpublished, to determine the optimum conditions for obtaining maximum expressible juice with minimum loss in the apparatus. Different materials, including asbestos wool, felt mats, filter paper, shot, sponge rubber and flat rubber mats, airplane cloth, and bolting silk, were placed with the meat in the cylinder as an aid in separating the juice from the muscle tissue. Different periods of time of applied pressure and different temperatures of both meat and apparatus were also studied. The procedure finally developed consisted in grinding hot cooked meat, wrapping a 50-gm. aliquot in an 18-inch square of bolting silk, placing the sample between two thin rubber pads in the cylinder, and pressing at a total of 10,000 pounds for 5 minutes. Some study was also made of the relation of the juice expressed in this way to the scores of a palatability committee, but a significant correlation was not found.

From this review of the literature it is apparent that the juice of meat has been obtained by pressure and has been analyzed physically and chemically, but there is no indication that any of the methods replaces the judging of samples for juiciness by a palatability committee. It is the purpose of this paper to present a mechanical method for determining the juice content, or juiciness, of meat, that is believed to possess certain advantages over the technique now available for measuring this factor.

It was the opinion of the authors that in the mechanical method the technique of testing and the conditions under which it takes place should not only yield consistent results but should also be easy to duplicate. In view of the fact that the investigator may wish to study the closely related factor of character of juice, all the extracted juice should be so collected as to be available for examination. The method should measure the same characteristics as are measured by human judges, but the results should be more dependable. The proposed method, which was developed by the authors at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., is described in this paper.

DESCRIPTION OF PROPOSED METHOD

APPARATUS

Figure 1 shows the hydraulic laboratory press used in the proposed method for determining the juiciness of meat. This instrument is of standard make, equipped with electrically heated plates, and capable of producing up to 20,000 pounds of pressure. The steel test cylinder is 2¼ inches in internal diameter and 3 inches in depth. A cross section of the cylinder showing the working mechanism is given in figure 2. In the meat laboratory of the Bureau of Animal Industry a Babcock milk-testing bottle is used as the receiver. By the use of this bottle the estimation, if desired, of the fat content of the juice by a modified Babcock technique is facilitated.

The following modifications of the test cylinder, as purchased, were found to be necessary: (1) Filling with solder the two grooves in the bottom of the cylinder; (2) fitting the base piece with a removable steel tube (figs. 1 and 2) 7½ inches long and ⅛ inch in outside diameter; and (3) adding a support for the base, this support being a steel disk 2½ inches in diameter and 1 inch thick. Other necessary apparatus are a disk of packing rubber 2$\frac{5}{16}$ inches in diameter and ⅛ inch thick and a disk of surgeon's gauze 2½ inches in diameter.

SAMPLING

After the roast is removed from the oven, it is allowed to remain at room temperature for 10 to 15 minutes. Samples are then taken as follows:

In beef, the muscle to be tested is dissected from the rest of the meat and cut perpendicular to the fibers midway between the ends. On each half of the muscle another cut is made parallel to and 1 inch from the cut surface. From each of the two resulting slices a cylindrical section of meat is taken perpendicular to, and from the center

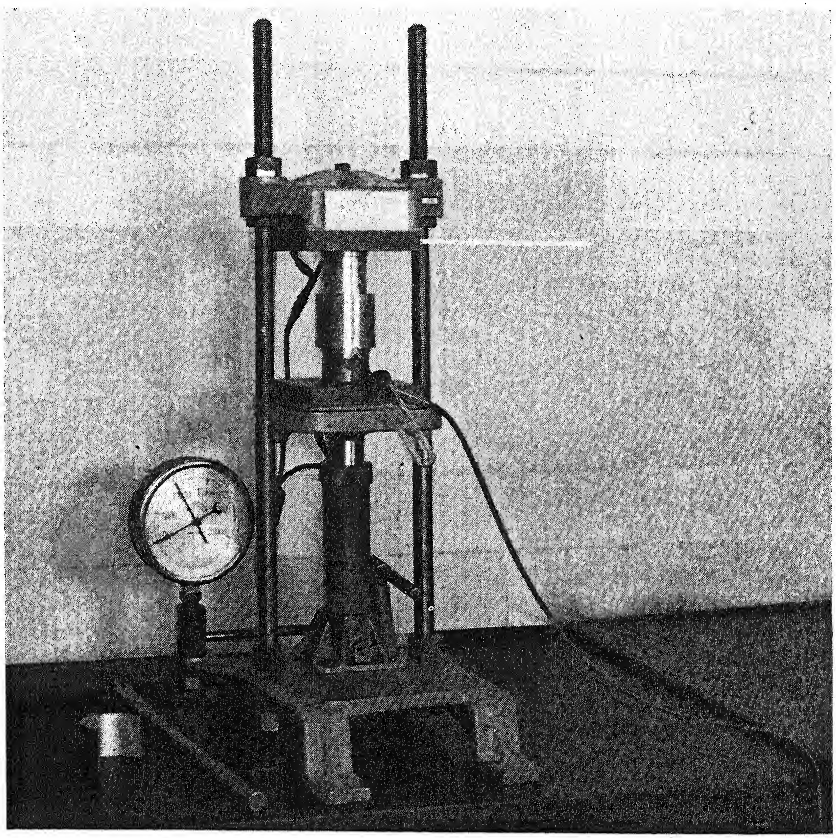

FIGURE 1.—Hydraulic press used in the determination of the expressible juice content of meat.

of, the cut surface with a metal borer having an inside diameter of 1¾ inches. These two cylinders of meat are the samples, in duplicate. Each weighs about 36 gm. Both may be pressed, or one may be pressed and the other sliced for judging by a committee for comparative purposes.

For pork, the procedure is the same as for beef, but owing to the smaller size of the muscles the inside diameter of the borer is 1 inch and the length of the sample is 2 inches. The weight is approximately 23 gm.

In leg of lamb, a cut is made through the popliteal gland as in the standard method of slicing for judging. Another cut is made parallel to this and three-fourth inch toward the pelvic bone, and the slice is then removed from the leg. With the 1-inch borer three pieces are taken from the semimembranosus muscle at positions of uniform distribution in this slice and used as one sample. This sample weighs about 23 gm. The part of the semimembranosus muscle remaining attached to the pelvic bone may be used for committee judging. In the rib and loin of lamb, owing to the small diameter of the muscle, the borer cannot be used. The entire muscle is dissected, trimmed of fat and connective tissue, cut through the center, and a slice 1 inch

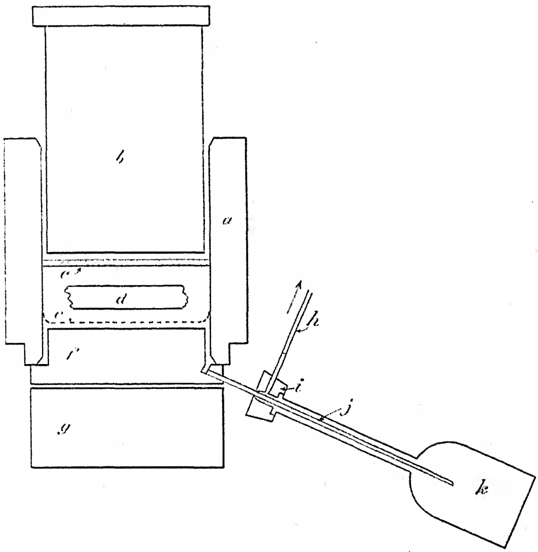

FIGURE 2.—Test-cylinder apparatus in cross section, showing *a*, cylinder; *b*, plunger; *c*, rubber disk; *d*, meat sample; *e*, gauze disk; *f*, base; *g*, support; *h*, suction tube; *i*, rubber stopper; *j*, collecting tube; *k*, receiver.

thick from each half is taken, one being used for pressing and the other for committee judging.

All samples, as soon as taken, are placed in aluminum moisture dishes and kept in an electric oven at 45° to 50° C. until ready for the press.

PRESSING

The hot plates and test-cylinder apparatus are heated to a temperature of 50° C. for the test. The cylinder is placed on the base and the gauze disk inserted (fig. 2). The sample of meat is placed in the cylinder, the rubber disk tucked in over it, and the plunger inserted. The receiver is connected to the tube and the assembled outfit placed in the press on top of the metal disk in order to clear the tube (fig. 2). The connection of the receiver to the source of suction is by means of a tube. The suction is turned on just enough to prevent the leakage of juice around the base of the cylinder. Pressure is applied very slowly.

Juice flows profusely into the receiver until the gage registers about 2,500 pounds. From this point on the pressure is so increased that the juice trickles out at the rate of 1 drop every 2 or 3 seconds, until a pressure of 9,800 pounds is reached. The suction is increased slightly and the pressure maintained at 9,800 pounds (approximately 2,500 pounds per square inch of cylinder area) for 5 minutes. Then the pressure is released and the press cake removed from the cylinder. The loss in weight of the sample is taken as the weight of expressed juice.

ACCURACY OF METHOD

In the development of this method, use was made of numerous pairs of samples that were believed to be as nearly alike in juice content as could be obtained. These samples were taken, for example, from the same muscle or the corresponding muscles from the left and right sides of the carcass. As evidence of the small error involved in the method, part of which undoubtedly is due to sampling, there are given in tables 1 and 2 data from paired four-vertebrae roasted pork-loin samples from 18 animals. In table 1, differences between paired adjacent samples ranged from 0 to 9.9 percent and averaged 2.4 percent. In the paired samples from the left and right sides of carcasses (table 2) the differences ranged from 0.1 to 9.6 percent and averaged 2.9 percent. According to the t test of Fisher (7), neither the difference between means in table 1 nor that in table 2 is significant.

In table 1, only two of the differences between adjacent samples exceed 5 percent, whereas in table 2, involving samples from left- and right-side cuts, there are eight such differences. This situation seems logical since it is more probable that adjacent samples of meat from the same muscle will be in the same physical condition than samples of corresponding muscles from the left and right sides of the carcass. Any variation in heat penetration, for example, would be a less disturbing influence in the former.

TABLE 1.—*Expressible juice content of adjacent roasted pork-loin samples tested by the proposed method*

Sample pair No.	Juice content of—		Difference in juice content between samples	Sample pair No.	Juice content of—		Difference in juice content between samples
	Sample *a*	Sample *b*			Sample *a*	Sample *b*	
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	30.9	28.5	2.4	19	40.9	34.5	6.4
2	29.6	26.3	3.3	20	42.6	32.7	9.9
3	30.2	28.7	1.5	21	34.5	38.0	3.5
4	33.6	32.0	1.6	22	41.1	40.7	.4
5	37.5	37.5	0	23	37.8	35.7	2.1
6	36.8	33.8	3.0	24	38.1	35.9	2.2
7	32.0	32.8	.8	25	37.4	32.6	4.8
8	32.4	31.3	1.1	26	43.0	38.4	4.6
9	30.0	33.6	3.6	27	32.7	34.7	2.0
10	37.0	36.9	.1	28	33.6	28.8	4.8
11	40.0	37.9	2.1	29	35.2	34.8	.4
12	38.2	39.2	1.0	30	33.2	34.4	1.2
13	35.7	33.8	1.9	31	37.3	34.6	2.7
14	34.2	33.7	.5	32	40.9	41.3	.4
15	39.2	39.6	.4	33	40.0	37.2	2.8
16	39.3	38.9	.4	34	35.4	33.8	1.6
17	39.6	37.7	1.9	35	29.7	31.7	2.0
18	33.0	28.1	4.9	36	28.2	33.0	4.8
				Mean	35.9	34.5	2.4

TABLE 2.—*Expressible juice content of samples from corresponding left and right roasted pork loins listed in table 1*

Sample pair No.	Juice content of—		Difference in juice content between samples	Sample pair No.	Juice content of—		Difference in juice content between samples
	Left loin	Right loin			Left loin	Right loin	
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	30.9	29.6	1.3	19	40.9	42.6	1.7
2	28.5	26.3	2.2	20	34.5	32.7	1.8
3	30.2	33.6	3.4	21	34.5	41.1	6.6
4	28.7	32.0	3.3	22	38.0	40.7	2.7
5	37.5	36.8	.7	23	37.8	38.1	.3
6	37.5	33.8	3.7	24	35.7	35.9	.2
7	32.0	32.4	.4	25	37.4	43.0	5.6
8	32.8	31.3	1.5	26	32.6	38.4	5.8
9	30.0	37.0	7.0	27	32.7	33.6	.9
10	33.6	36.9	3.3	28	34.7	28.8	5.9
11	40.0	38.2	1.8	29	35.2	33.2	2.0
12	37.9	39.2	1.3	30	34.8	34.4	.4
13	35.7	34.2	1.5	31	37.3	40.9	3.6
14	33.8	33.7	.1	32	34.6	41.3	6.7
15	39.2	39.3	.1	33	40.0	35.4	4.6
16	39.6	38.9	.7	34	37.2	33.8	3.4
17	39.6	33.0	6.6	35	29.7	28.2	1.5
18	37.7	28.1	9.6	36	31.7	33.0	1.3
				Mean	35.1	35.3	2.9

One of the possible sources of error in comparing left and right samples after heating is the layer of external fat on the cut of meat. It is not unusual, after the back fat has been removed in the abattoir, to obtain a pair of pork-loin cuts one of which has a considerable and even layer of fat whereas the other has either a thinner fat layer or an uneven one, muscle being exposed in some places. Furthermore, it is not always possible to obtain cuts that are of exactly the same size, shape, and weight.

EVALUATION OF METHOD

To represent an actual advance in technique, a mechanical method for measuring a palatability factor of meat must enable the investigator to obtain more dependable, significant data than can be obtained from the determinations of judges. However, it is necessary to evaluate the mechanical method by comparison with committee judging.

For such a comparison, 39 fresh beef-round samples, 30 fresh pork-loin samples, and 27 freezer-stored pork-loin samples were used. The cut of round of beef, 4 inches thick, was heated in an electric oven at 125° C. to an internal temperature of 58°. From each cut, three samples were taken, one from the semitendinosus and two from the semimembranosus muscle. Each of these samples was divided into two parts. One-half furnished the slices that were judged by a committee of four to six persons, and the other was used to determine the expressible juice content by the mechanical method. The pork loins were heated at 150° C. to an internal temperature of 84°. In a manner similar to that employed with beef, a section of the longissimus dorsi muscle was removed and one-half was used for the judges, while the other half furnished a sample for the determination of expressible juice content.

Samples were scored by the judging committee as follows: 5, juicy; 4, moderately juicy; 3, slightly juicy; 2, slightly dry; 1, dry. For each sample the scores of the several judges were averaged to give a

committee score. For the beef samples the committee scores ranged from 2.3 to 4.3 and the expressible juice content from 29.3 to 55.3 percent. For the fresh-pork samples the corresponding ranges were 1.0 to 3.8 and 35.9 to 50.0, and for freezer-stored pork they were 1.5 to 3.5 and 34.9 to 50.2.

The coefficients of correlation between committee scores and the percentages of expressible juice for the three groups of samples were as follows: Fresh beef, $+0.63 \pm 0.07$; fresh pork, $+0.60 \pm 0.08$; freezer-stored pork, $+0.58 \pm 0.09$. The foregoing coefficients of correlation obviously are not high. However, they were regarded by the authors as of sufficient magnitude to be encouraging.

In considering the coefficients, attention was drawn to the relatively narrow ranges of committee score for juiciness and percentage of expressible juice. It was believed that with wider ranges of these factors a better measure would be obtained of the relation between them. Therefore an experiment was set up with a view to effecting wide differences in juiciness. One group of pork-loin cuts was heated to an internal temperature of 60° C., another to a temperature of 85°, and a third to 100°, at an electric-oven temperature of 150° in all instances. In the first group, containing 32 samples, the ranges in committee score and percentage of expressible juice were from 2.5 to 4.3 and 47.3 to 57.7, respectively. For the 18 samples in the second group the respective values were 1.5 to 3.5 and 35.7 to 48.0, and in the third group, containing 26 samples, the ranges were 1.0 to 2.8 and 24.7 to 42.5. For all 76 samples, the range in committee scores was from 1.0 to 4.3 and in expressible juice content from 24.7 to 57.7 percent. Similar procedures were applied to rib and loin cuts of beef and lamb.

Correlation coefficients were calculated and equations of the form $y=a+bx$ for estimating committee score (y) from percentage of expressible juice (x) were derived. Table 3 shows, for the three types of meat investigated, high coefficients of correlation, with little variation among them, and a wide range in committee score. The lamb meat varied as widely as the pork in juiciness, and the scores on beef covered the full range, from 1.0 to 5.0.

TABLE 3.—*Relation between (1) committee score for juiciness and (2) expressible juice content of three kinds of meat*

Kind of meat	Samples	Committee score (y)		Expressible juice (x)		Coefficient of correlation and probable error	Estimating equation	Standard error of estimate
		Range	Mean	Range	Mean			
	Number			*Percent*	*Percent*			
Pork loins	76	1.0–4.3	2.73	24.7–57.7	43.29	0.89 ± 0.01	$y=0.089x-1.129$	0.326
Beef ribs and loins	68	1.0–5.0	3.60	17.7–55.0	41.49	$.92 \pm .01$	$y=.113x-1.070$	.437
Lamb ribs and loins	54	1.0–4.3	2.94	18.5–51.8	38.22	$.85 \pm .02$	$y=.084x-.256$	.398

In this comparison of the percentages of expressible juice and committee scores, it was found that pork, beef, and lamb having the same percentages of expressible juice were judged by the committee to differ considerably in degree of juiciness as perceived by taste (table 4). In most instances, as shown by the table, with a given percentage of expressible juice, beef was rated by the judges as being the most

juicy and pork the least. For instance, pork containing 40 percent of expressible juice was judged to be only slightly more juicy than beef or lamb containing 30 percent of expressible juice. Beef containing 45 percent of juice and lamb containing 50 percent were considered by the judges to be practically of equal juiciness. Thus the kind of meat was found to influence human perceptions of juiciness.

TABLE 4.—*Committee scores, for estimated juiciness, of pork, beef, and lamb corresponding to indicated percentages of expressible juice*

Percent of expressible juice	Committee scores for estimated juiciness of—			Percent of expressible juice	Committee scores for estimated juiciness of—		
	Pork loins	Beef ribs and loins	Lamb ribs and loins		Pork loins	Beef ribs and loins	Lamb ribs and loins
25	1.1	1.7	1.8	40	2.4	3.4	3.1
30	1.5	2.3	2.3	45	2.9	4.0	3.5
35	2.0	2.9	2.7	50	3.3	4.6	3.9

SUMMARY

A mechanical method for determining the juiciness of cooked meat has been developed at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. It involves the use of a specially adapted hydraulic laboratory press for determination of the expressible juice content of samples weighing approximately 25 to 35 gm. Pressure on the sample in the test cylinder of the press, maintained at 50° C., is increased gradually to a total of 9,800 pounds and held there for 5 minutes. The juice is collected in a receiver, which may be a Babcock milk-testing bottle, thus facilitating subsequent determination of the fat content, if desired, by means of a modified Babcock technique. The difference in weights of the sample before and after pressing represents the quantity of expressible juice.

In a study of the accuracy of the new method, it was found that adjacent samples from the same muscle and samples from the corresponding muscles of the two sides of the carcass were not significantly different in expressible juice content. Moreover, the coefficients of correlation between committee scores and the expressible juice content of samples of beef that varied narrowly in juiciness, although not high, were of sufficient magnitude to be encouraging. The statement applies also to expressible juice of both fresh and freezer-stored pork. When wider ranges in scores for juiciness of fresh pork and beef were obtained by heating to widely varying internal temperatures, the relationships between such scores and the percentage of expressible juice were close. Likewise a close relationship was found in fresh lamb.

The results indicate that, for the most part, when samples of meat having the same percentage of expressible juice were judged by the committee, beef was rated as being the most juicy and pork the least.

The proposed method appears to be adaptable for use at least with beef, lamb, and pork when the variation in juiciness is due to animal production factors or to the internal temperatures to which the meat is heated. Possibly investigators will find it useful first as a supplement to and later as a replacement of the technique based on committee scores.

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EFFECTIVENESS AGAINST THE CALIFORNIA RED SCALE OF CUBE RESINS IN LIGHT-MEDIUM AND HEAVY SPRAY OILS[1]

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INTRODUCTION

An important characteristic used in grading mineral oils is their heaviness or volatility as indicated by their distillation range. It is generally agreed that the lighter, or more volatile, spray oils are less injurious to citrus trees than the heavy oils, but that they are less effective against the California red scale (*Aonidiella aurantii* (Mask.)) *5, 7*).[2] Rohrbaugh's finding (*6*) that the lighter oils disappear from citrus foliage more rapidly than the heavy oils indicates that the greater tolerance of citrus trees to the lighter oils is due, in part at least, to their more rapid evaporation from the foliage.

In a previous paper (*3*) it was shown that the effectiveness of heavy spray oils against the California red scale on lemons could be increased by combining nicotine or cube resins with the oil. Such results suggested the possibility of obtaining satisfactory control of the scale by means of the lighter oils combined with toxicants. Since from the standpoint of cost, toxicity, and possible phytocidal effects, cube was considered the more promising toxicant with heavy oil, experiments were designed to compare effectiveness of sprays of a light-medium and a heavy petroleum oil containing cube resins. Tests were made in both laboratory and field.

OIL SPECIFICATIONS

The specifications of the oils and their approximate distillation ranges are given in table 1.

Table 1.—*Specifications and distillation ranges of the oils used in the experiments*[1]

Oil	Viscosity, Sabolt, at 100° F.	Specific gravity at 20° C.	Unsulfonatable residue	Iodine No. (Hanus)	Acid value
			Percent		
Heavy	91	0.855	96	4.1	0.1
Light-medium	77	.872	100	2.6	.1

Oil	Proportion distillable at temperature (° F.) of—									
	560	575	600	625	636	650	675	700	725	750
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy	--------	--------	--------	--------	5	15	37	61	79	90
Light-medium	5	15	32	49	56	63	76	85	90	--------

[1] Specifications of the oils were determined by the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine. Data on the distillation ranges were furnished by the manufacturer.

[1] Received for publication August 4, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 419.

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FIELD EXPERIMENTS

The methods of preparation of emulsions, application of sprays in the field, and measurement of oil deposits were similar to those previously described (*3*). The oil concentration was 1.5 percent in all treatments. The cube resins, which contained 22.3 percent of rotenone, were dissolved in an intermediary solvent consisting of 1 part by volume of trichloroethylene and 2 parts of dibutyl phthalate and 10 volumes of this solvent was added to 90 volumes of oil before emulsification. This gave a concentration of 1 gm. of cube resins to 4 liters of spray. Four sprays containing light-medium and heavy oils, with and without cube resins, were applied on October 20 in a lemon grove rather heavily infested with the California red scale. Each treatment was applied to a plot of four trees.

OIL DEPOSITS

Each sample analyzed for oil deposit was made up of 100 leaf disks 1.5 cm. in diameter, 4 disks being taken from each of 25 leaves. Eight or nine samples were collected from each plot. The samples were taken on October 21, 24, and 25. It was found that deposits on the samples taken on the last 2 dates averaged lower than those taken on the first date, the difference being 8 percent for the heavy oil and 30 percent for the light-medium oil. This probably represents the amount of oil lost by volatilization. The values obtained on the later dates were corrected accordingly, and the average oil deposits represent estimates of the oil on the foliage the day after spraying. Deposits of heavy and light-medium oil were 141 and 149 micromilliliters per square centimeter, and deposits of the same oils with cube resins, 147 and 135 micromilliliters per square centimeter. The differences between deposits were not significant.

INSECT MORTALITY

Insect counts were limited to the more resistant females; namely, those in the late gray and older stages. Natural mortality in these stages, excluding those scales judged to have been dead before application of the spray, averaged about 5 percent and has been disregarded.

Scales on Wood

As there was not sufficient encrusted material on the twigs to include this type of infestation in the wood counts, the classes of scale densities examined were limited to light and heavy infestations on green and gray wood (*3*). Results of the scale counts made 5 to 6 weeks after spraying are shown in table 2.

Table 2.—*Mortality of California red scales on lemon wood sprayed with heavy and light-medium oil, with and without cube resins*

Kind of wood	Infestation	Heavy oil		Heavy oil+ cube		Light-medim oil		Light-medium oil + cube	
		Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	Total scale	Mortality
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Green	Light	718	88.0	504	99.6	771	78.5	486	99.6
	Heavy	767	77.2	782	95.8	762	69.0	902	97.2
Gray	Light	650	78.5	673	95.4	669	68.5	735	96.5
	Heavy	1,542	58.1	2,240	91.2	1,433	51.6	2.140	92.9
Average			75.5		95.5		66.9		96.6

In the analysis of these results the scale counts from each tree were taken as the sampling unit. No replication of plots seemed necessary, since the insects present at the time of treatment were still there when mortality counts were made, and the principal sources of variation in mortality, such as differences in oil deposits, developmental stages of scales involved, population density, type of host surface, and natural mortality, had been controlled or measured by the technique used.

Although the differences in effect between the heavy and light-medium oils were small, analysis showed that they were highly significant ($P<0.01$). When cube resins were added, there was a large increase in mortality and no difference between the effects of the two combinations.[3] Cube resins were just as effective in a light-medium oil as in a heavy oil in spite of the fact that when used alone the heavy oil was more effective. The effect of the oil-cube treatments upon scales in the heavy infestation on the gray wood was outstanding, as it was in the previous experiments reported (*3*).

Scales on Fruit

Mortality of scales on the fruit was determined about 4 weeks after spraying, by examining from 18 to 22 lemons given each treatment. The results are shown in table 3.

Table 3.—*Mortality of scales on lemon fruits*

Treatment	Total scales	Mortality
	Number	*Percent*
Heavy oil	3,878	88.3
Heavy oil plus cube resins	2,224	95.4
Light-medium oil	3,457	82.5
Light-medium oil plus cube resins	3,079	97.3

To permit statistical analysis of the differences between treatments, population densities were calculated, as described in the previous paper (*3*), and were found to range from 0.1 to 8.9 scales per square centimeter.

A trend toward lower mortality at the higher densities of infestation was evident only in the treatments in which cube was not used. The difference between the treatments with heavy and light-medium oils was not statistically significant.[4] However, the difference was in the same direction as that between the counts of scales on the wood and may mean that the heavy oil was slightly more effective against the scales on the fruit even though the differences cannot be shown to be significant. Both oil-cube sprays were superior to sprays containing the same oil without cube. The difference in mortality between the two oil-cube sprays was not significant.

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[3] Since all the mortalities following treatments with oil plus cube were above 85 percent, the inverse sine transformation was made before computing the variance (*2*). This transformation was not used in the analysis of the treatments with oil alone.

[4] The test of significance was made as follows: Population density-mortality regression lines were fitted to the data by the method of least squares after each percentage had been weighted by the reciprocal of its variance. The difference between the means was then tested for significance by computing t from the formula.

$$t=\frac{(a_1-a_2)-b_c(\bar{x}_1-\bar{x}_2)}{\sqrt{V(a_1)+V(a_2)+(\bar{x}_1-\bar{x}_2)^2V(b_c)}}$$

where a_1 and a_2 denote the mean percentages dead, x_1 and x_2 the mean densities per square centimeter for treatments with heavy and light-medium oil, respectively, and b_c is a combined regression coefficient for the two treatments (*1*).

LABORATORY EXPERIMENTS

The cube resin used in the laboratory tests contained 27.4 percent of rotenone. It was dissolved in a mixture of trichloroethylene and dibutyl phthalate, as in the field tests. Emulsions were prepared with a high-speed drink mixer and applied with a precision sprayer (fig. 1)

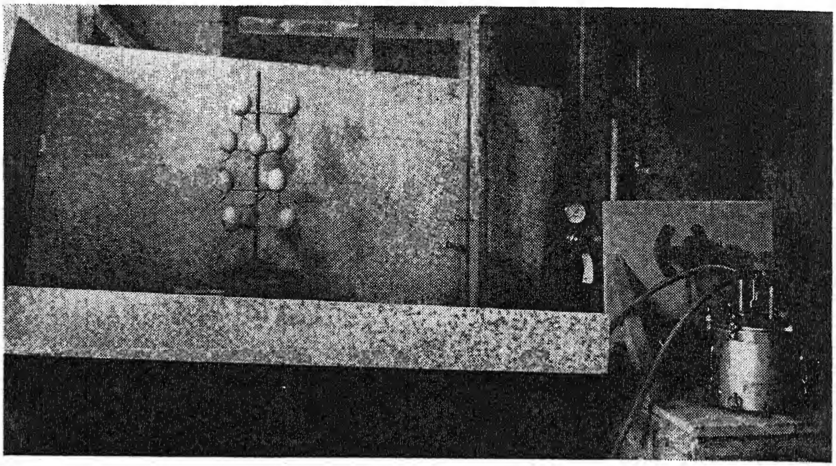

FIGURE 1.—Laboratory equipment used for applying oil sprays on lemons.

similar to that described by Dawsey, Cressman, and Hiley (*4*). Lemons were rested on circular wire supports soldered to a laboratory ring stand, which was mounted on an electrically driven turntable. Each set of lemons was sprayed for 45 seconds at a pressure of 35 pounds per square inch, while the nozzle was moved up and down in a vertical plane and the turntable revolved at the rate of 20 revolutions per minute.

The lemons were infested with scales that had been reared in the laboratory at 25° C. by a modification of a method described by Munger.[5] They were sprayed shortly before the scales began to reproduce, and counts were limited to females in the late gray and older stages, most of them being in the mature stage.

As a result of the method of infestation of these lemons and a rough selection before treatment, it was possible to restrict the range of population densities within rather narrow limits in a given series of spray tests, so that the average mortalities, which are shown in table 5, make a valid basis for comparison of the sprays applied at the same time.

[5] MUNGER, F. A METHOD FOR INFESTING LEMON FRUIT WITH RED SCALE. U. S. Bur. Ent. and Plant Quar. Cir. ET120, 3 pp., illus. 1938. [Processed.]

TABLE 5.—*Effect of adding cube resins to laboratory sprays of light-medium and heavy oils on the mortality of the California red scale* [1]

Spray	Treatments of Jan. 27, oil concentration, 1.5 percent		Treatments of Mar. 15, oil concentration 1.0 percent	
	Total scales	Mortality	Total scales	Mortality
	Number	*Percent*	*Number*	*Percent*
Light-medium oil	1,993	55.4	1,633	57.4±3.01
Heavy oil	1,862	76.3	1,365	61.7±2.84
Light-medium oil plus cube resins	2,000	99.2	1,171	98.0±0.51
Heavy oil plus cube resins	1,781	98.6	1,632	93.4±0.85

[1] Standard errors of the mortalities resulting from lemons sprayed Jan. 27 were not calculated, since it was evident from an inspection of the data that the difference between the first pair of treatments was significant and the difference between the two sprays containing cube was not significant. In the treatments of March 15 the fruits were used as the sampling units in computing the standard errors.

In the first sprays, which were applied January 27 and contained 1.5 percent of oil, the heavy oil was more effective than the light-medium oil when used alone, but when cube resins were added at the rate of 1 gm. to 5 liters of spray there was no difference in their effectiveness. The results of the first laboratory sprays, therefore, were corroborative of the field results, although the differences between treatments were more marked.

In both the field and the first laboratory experiments the mortality from light-medium oil plus cube was slightly higher than that from heavy oil plus cube, but the difference was not statistically significant. The fact that the differences were in the same direction in all cases suggests the possibility that there were slight but real differences in the effectiveness of the two combinations which could not be demonstrated from these two experiments.

Since variability due to uneven coverage and other factors in field sprays might be expected to obscure small differences in efficiency, and the mortality in both oil-cube treatments applied in the laboratory was too high to favor the detection of small differences, the sprays applied March 15 were designed to permit a more satisfactory comparison of the heavy and light-medium oils containing cube resins. Oil concentrations were lowered to 1.0 percent, and the concentration of cube resins was lowered to 1 gm. to 6 liters. In these tests the difference in mortality between the heavy and light-medium oils used without toxicants was less than in the earlier tests, but when cube was added the difference in favor of the light-medium oil was highly significant. The laboratory tests of Marcy 15 were accompanied by measurements of oil deposits on bottles coated with beeswax. No large differences in the oil deposits were found.

DISCUSSION

There are several possible reasons for the greater effectiveness of cube resins in the lighter oil. More of the light-medium oil may have gotten beneath the scale covering, in which case the dosage of cube per scale would have been higher than in the tests with the heavy oil. Furthermore, the light-medium oil may have reached the insect more quickly than the heavy oils. Since cube gradually loses toxicity when exposed to light and air on the plant, a quicker contact with the insect

would be expected to make for greater effectiveness. The possibility of a greater solubility of the toxic principles of the resins in the particular light-medium oil used cannot be entirely ignored. There was no evidence that differences in the oil deposits were a factor in these tests.

In these experiments where efficiency was measured by the kill of the older female scales, one important effect of oil sprays has not been considered, viz, the residual effect of the oil film in inhibiting the settling of young produced by the survivors. It seems probable that the heavier oils, which leave a more persistent residue, would be superior to the lighter oils in this respect. Consequently, the total effectiveness of the light-medium oils and cube resins may not be so great as that of the heavy oils and cube resins, even though a greater increment in effect on the adult scales results from addition of the resins to the lighter oils.

SUMMARY

The relative effectiveness against the California red scale (*Aonidiella aurantii* (Mask.)) of sprays of cube resins in heavy and light-medium petroleum oils has been studied. The cube resins were dissolved in an intermediary solvent consisting of 1 volume of trichloroethylene and 2 volumes of dibutyl phthalate. Insecticidal efficiency was determined on the basis of the mortality of females in the gray adult and older stages. Counts were made on fruit and wood in the field tests, on fruit only in laboratory tests.

In the field tests all sprays contained 1.5 percent of oil. Cube resins were used at the rate of 1 gm. of the resins, containing 22.3 percent of rotenone, to 4 liters of diluted spray. A heavy oil without cube gave a more effective spray than a light-medium oil alone, but a light-medium oil with cube was just as effective as a heavy oil with cube. Mortality on the heavily infested older wood was 51.6 percent with light-medium and 58.1 percent with heavy oil. It was increased to 92.9 and 91.2 percent by the addition of cube resins.

The first laboratory sprays contained 1.5 percent of oil and cube resins, with a rotenone content of 27.4 percent, at the rate of 1 gm. to 5 liters. Mortality of 55.4 and 76.3 percent from a light-medium and a heavy oil was increased to 99.2 and 98.6 percent by the addition of cube. In a second series of laboratory sprays containing 1.0 percent of oil and 1 gm. of cube resins to 6 liters of spray, the differences between the effects of the light-medium and heavy oils without toxicants were less than in the preceding tests, but light-medium oil plus cube was more effective than heavy oil plus cube, mortality being 98.0 and 93.4 percent, respectively.

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THE EFFECT OF DIFFERENT OXYGEN CONCENTRATIONS ON THE RATE OF RESPIRATION OF AZOTOBACTER IN RELATION TO THE ENERGY INVOLVED IN NITROGEN FIXATION AND ASSIMILATION [1]

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INTRODUCTION

It has been shown (*7*,[3] *fig. 9*) that during nitrogen fixation by *Azotobacter* at 20 percent oxygen the reaction $C_6H_{12}O_6+6O_2=6CO_2+6H_2O \Delta H=-674{,}000$ calories is one reaction that takes place at a constant rate for a considerable period of time when adequate oxygen is supplied and when the end product (carbon dioxide) is removed from the culture as fast as formed. If *Azotobacter* may be considered as a catalyst for this reaction, the rate at which the reaction proceeds (within reasonable limits) should be a function of the partial pressure of oxygen.

METHODS

Studies on the respiratory rate of *Azotobacter* at different partial pressures of oxygen, while fixing nitrogen and also while assimilating combined nitrogen, were carried out with the apparatus and technique described earlier (*7*). The different partial pressures of oxygen were obtained by filling a series of 12 tanks to 1,000 pounds pressure with a mixture of pure oxygen and nitrogen. The concentration of oxygen in each tank was then accurately determined by analysis. It was thus possible to subject the culture to any one of the 12 different partial pressures of oxygen by connecting the proper tank (*7, fig. 2,A*) to the air-conditioning apparatus.

RESULTS

RESPIRATORY RATE OF AZOTOBACTER AT DIFFERENT PARTIAL PRESSURES OF OXYGEN

WHILE FIXING NITROGEN

Twelve hundred grams of a 36-hour culture at pH 7.2 (prepared as previously described (*7*)) was transferred aseptically to the sterile calorimeter. A gas mixture containing 17.8 percent oxygen was immediately started through the system at the rate of 14 liters per hour. The measurements were not begun, however, until the rate of respiration as measured by both the heat and CO_2 production became constant, thus insuring a constant number of living organisms. The

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[2] The author is indebted to G. N. Lewis, Merle Randall, D. R. Hoagland, A. R. Davis, and C. B. Lipman, of the university staff, and to F. C. Steward, of the Department of Botany, Birkbeck College, University of London, for interest shown in the work and for valuable suggestions and criticisms.

[3] Italic numbers in parentheses refer to Literature Cited, p. 440.

rate of heat and CO_2 production was then studied at six different partial pressures of oxygen lower than the partial pressure of oxygen in air. At each concentration, the rate of respiration was observed continuously for 10 to 18 hours. Starting with 17.8 percent oxygen and descending to 0.5 percent, each of the six partial pressures was studied in turn. As soon as the rate of respiration with the lowest oxygen concentration was determined, the higher concentrations in an ascending order were again applied and the respiration rates were

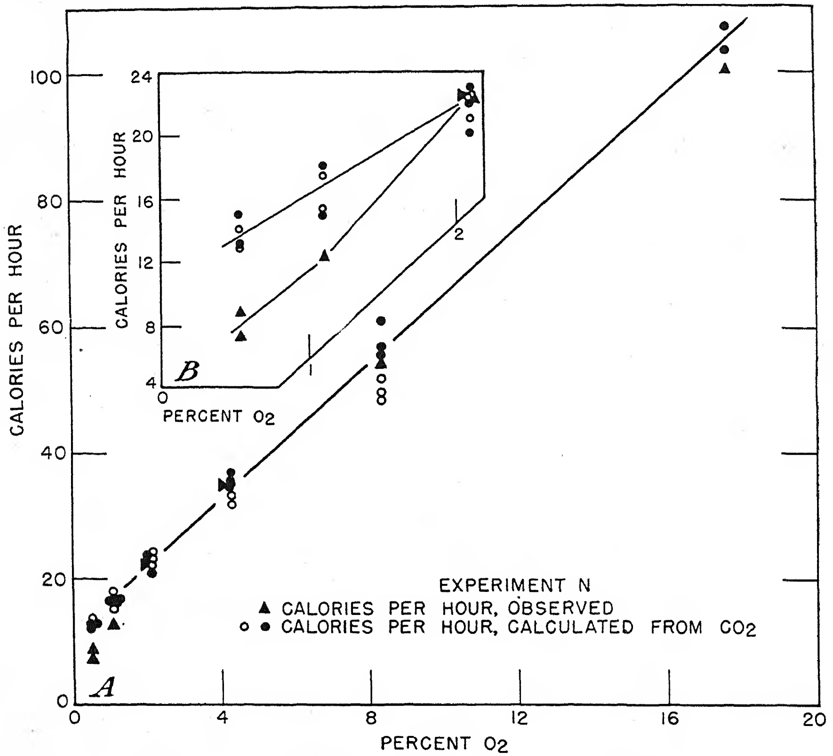

FIGURE 1.—The rate of respiration of *Azotobacter* (while fixing nitrogen) in the presence of six different partial pressures of oxygen less than that in air, applied first in a descending order and then in an ascending order. *A*, Calories per hour descending from 17.8 to 0.5 percent oxygen. *B*, Calories per hour ascending from 0.5 to 8.3 percent oxygen.

determined. In this way, the rate of respiration at each partial pressure of oxygen was approached from both extremes.

Figure 1 shows clearly the response made by the culture when subjected to the six different partial pressures of oxygen. The rate of respiration varied almost directly with the partial pressure of oxygen throughout the range studied. In the four higher concentrations less than 1 percent of the available oxygen was utilized, while in the lower concentrations the value rose to approximately 1.5 percent. Because of the enormous gas-liquid interface, and the amount of oxygen available, which was about 100 times that used, the cells did not suffer from lack of oxygen. To make sure of this, the rate of air flow was

increased 50 percent. This, however, did not increase the rate of respiration at any oxygen concentration, in spite of the 50 percent increase in available oxygen and a corresponding increase in the gas-liquid interface.

It has been shown (*7, fig. 8*) that if the rate of air flow is decreased sufficiently, depending upon the oxygen concentration, a decrease in the rate of respiration will result. However, this decrease may be due only in part to a lack of oxygen, for under such conditions carbon dioxide would accumulate in the medium, thus causing an increase in the hydrogen-ion concentration and other unfavorable conditions. When the gas-liquid interface was increased by increased rate of aeration (which would result in a more rapid gaseous exchange), a maximum rate of respiration was reached.

Figure 1, *B*, clearly shows that *Azotobacter* will respire with a respiratory quotient well above unity if the oxygen concentration is reduced below 2 percent.

The objection may be raised that the number of living organisms decreased in the same proportion as the partial pressure of oxygen was decreased. It must be remembered, however, that the total change in the rate of respiration occurred within less than 15 minutes after the partial pressure of oxygen was altered. After this sudden shift in concentration, the rate of respiration remained constant until the partial pressure of oxygen was again changed (see *7, fig. 6*). It is not reasonable to assume that the required number of organisms would die within such a short time in order to effect the observed decrease in the rate of respiration.

To demonstrate again that the number of living organisms did remain constant and were at a steady state regardless of the partial pressure of oxygen used, the rate of respiration was studied with increasing partial pressures of oxygen as described. Proceeding from the low partial pressure of oxygen to the higher, the total increase in the rate of respiration occurred within 15 minutes after the higher oxygen concentration reached the culture. Here, again, to account for this sudden increase in rate of respiration by an increase in the number of living organisms, it would be necessary to assume that the culture almost doubled its number within 15 minutes.

Twenty hours after the beginning of the experiment the rate of respiration with 8.3 percent oxygen was found to be 56 calories per hour. The rate of respiration with this same concentration of oxygen was again studied 110 hours later, under the same conditions, but after the partial pressure of oxygen had undergone nine changes. In spite of such changes in the oxygen concentration and the lapse of 110 hours, the rate of respiration was 51 calories per hour. The respiration rates at 40, 70, and 110 hours with 2.1 percent oxygen were found to be 22, 23, and 25 calories per hour respectively. Here, again, the rate of respiration is the same for a given oxygen concentration and is not affected by the previous oxygen treatment. At 50, 80, and 100 hours, the rate of respiration was 17, 17, and 15.5 calories per hour, respectively, with 1.1 percent oxygen. One hundred and thirty hours after the beginning of the experiment the final observation was made on the rate of respiration at 8.3 percent oxygen (fig. 1). Pure oxygen was immediately added and the respiratory rate was determined 45 minutes later. The rate of respiration as measured by both the CO_2 and heat production was found to increase from 56 to 245 calories

per hour. In other words, there was a 338 percent increase in the rate of respiration within 45 minutes (and very likely within 15 minutes, had readings been made) after the oxygen tension was increased from 8.3 to 100 percent. It is hardly reasonable to assume that the living

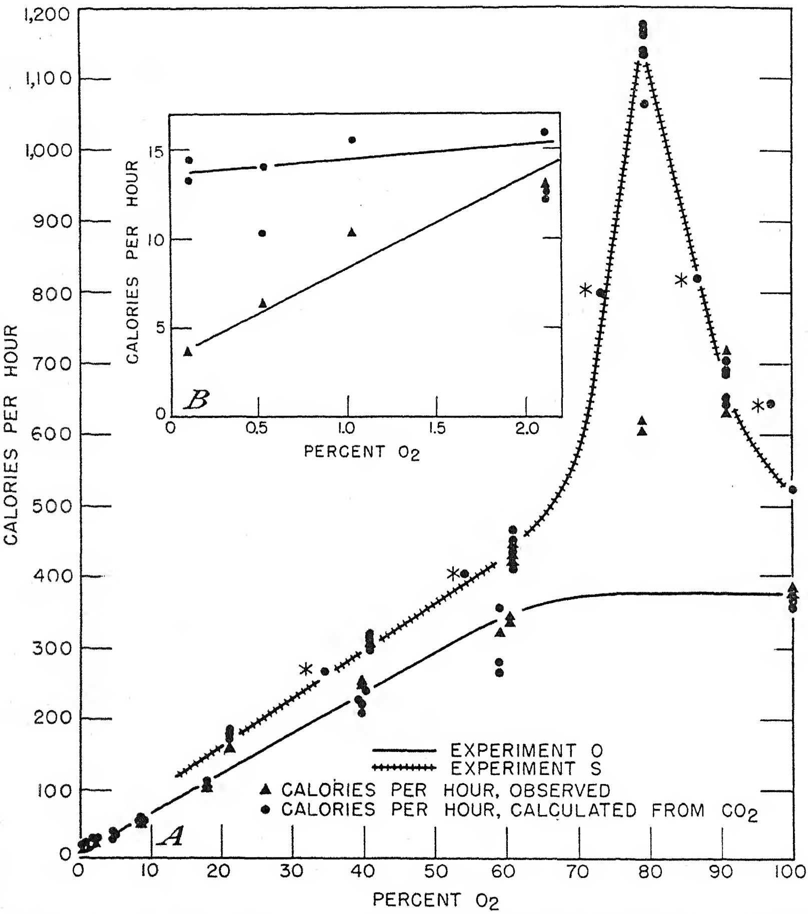

FIGURE 2.—The rate of respiration of *Azotobacter* (while fixing nitrogen) subjected to partial pressures of oxygen covering the entire range. The points designated by the asterisk represent the CO_2 evolved during 1 hour, the oxygen tension remaining at the lower value for 20 minutes and at the higher value for the remaining 40 minutes. The average tension is therefore the lower tension plus two-thirds the difference between it and the next higher oxygen tension. *A*, data from experiments O and S; *B*, the three lowest points in the data from experiment O plotted on a larger scale.

cells in the culture, subjected to so many different partial pressures of oxygen, could either increase or decrease in number in such a short time and at the same time be dependent only on the partial pressure of oxygen supplied.

To determine the effect of different oxygen concentrations above that in air on the rate of respiration and to confirm the results obtained

with partial pressures of oxygen less than that of air, the experiment was repeated. The culture was allowed to reach a steady state in 18 percent oxygen after which time the experiment was started with nitrogen containing 0.1 percent oxygen. The higher oxygen concentrations were in turn forced through the culture and the rate of respiration determined by both the heat and CO_2 produced. The results are shown in figure 2.

The results of this experiment confirm those of the previous experiment (fig. 1) over the lower range and show that the rate of respiration increases in the higher oxygen concentration up to 60 percent oxygen. Above this amount the curve appears to flatten out, but in no case does the rate of respiration decrease with increasing oxygen concentrations.

The two CO_2 points at 40 and 60 percent do not coincide with the heat measurements. It is possible that the rate of air flow was scarcely sufficient to prevent a slight accumulation of CO_2 in the medium. However, the heat measurement points fell on the curve, showing that this slight accumulation of CO_2 was not toxic. When 100 percent oxygen was added, the rate of aeration was increased from 12 to 14 liters per hour and under these conditions the rate of respiration, as calculated from the CO_2 evolved, became equal to the observed value.

To show again that the number of living organisms remained constant and that the same rate of respiration at these oxygen concentrations can be obtained by approaching from either the low or the high oxygen concentration limit, the oxygen tension was suddenly dropped from 100 to 60 percent and the rate of respiration was determined at an aeration rate of 14 liters per hour. After the effect of 60 percent oxygen was studied, 38 percent oxygen was substituted. In both cases the points fell on the curve (the highest carbon dioxide points at 40 and 60 percent oxygen). At these two points a 17 percent increase in the rate of aeration did not increase the observed rate of respiration above that obtained with the lower values for air flow. However, the calculated rate as determined by the carbon dioxide evolved was increased, but not beyond the observed rate.

Figure 2, *B*, shows the three lowest points of experiment O plotted on a larger scale. It is evident that more carbon dioxide is evolved than can be accounted for by the heat produced, which suggests that under these conditions *Azotobacter* is able to utilize combined oxygen to carry on respiration. In calculating the respiratory quotient (calories per hour calculated from the carbon dioxide evolved to the calories per hour observed) for *Azotobacter* subjected to different partial pressures of oxygen, it is evident (table 1) that in 0.1 percent oxygen this quotient is very high. A respiratory quotient of unity was not approached until the oxygen concentration had reached 2 percent.

In order to confirm the results obtained with the higher oxygen concentrations in experiment O, the experiment was repeated. In this experiment, the oxygen concentrations below that of air were omitted, but in order to define the upper portion of the curve more accurately 78.8 and 90.3 percent concentrations were also used.

TABLE 1.—*Respiratory quotient of Azotobacter while fixing nitrogen under different partial pressures of oxygen*

Oxygen (percent)	Experiment N			Experiment O		
	Average energy values per hour		Respiratory quotient (CO_2/O_2)	Average energy values per hour		Respiratory quotient (CO_2/O_2)
	Calculated from CO_2 evolved	Observed		Calculated from CO_2 evolved	Observed	
	Calories	*Calories*		*Calories*	*Calories*	
0.1				13.8	4.5	3.07
0.5	13.8	7.9	1.75	10.9	6.3	1.72
1.1	16.4	12.1	1.36	11.2	10.3	1.09
2.1	21.6	22.4	.97	13.6	13.1	1.03
4.3	35.6	34.7	1.03	34.1		
8.3	56.5	54.0	1.04	62.4	55.3	1.12
17.8	99.0	100.0	.99	105	109	.96
39.3				215	248	.87
58.8				278	322	.87
99.9				362	384	.94

The calorimeter was aseptically charged with a growing culture of *Azotobacter*, as previously described, and 21 percent oxygen was supplied until the rate of respiration reached a constant value. Each oxygen concentration was applied in turn and the rate of respiration measured continuously by both heat evolution and CO_2 absorption methods for 5 to 8 hours.

Results from experiment S, in figure 2, confirm those of experiment O and show again that the rate of respiration is dependent on the partial pressure of oxygen and that it is almost directly proportional to the oxygen concentration up to 60 percent. Both the heat produced and the heat that should have been liberated, as calculated from the CO_2 evolved, gave the same results. At 60 percent oxygen the rate of respiration continued to increase at the normal rate, while at 78 percent oxygen the rate (as measured by the CO_2 evolved) reached a maximum very abruptly. The observed rate of respiration did not increase sharply at this point.

It was further noted that at this latter oxygen concentration, only 53 percent of the energy liberated, as calculated from the CO_2 evolved, was found as heat. That this represents a critical point is evident from the fact that the rate of respiration remained constant at 78 percent oxygen for 4½ hours. The oxygen concentration was greater than 60 and lower than 90 percent for 6½ hours, as shown by eight CO_2 determinations. The points designated by the asterisk represent the rate of respiration due to the slight mixing of the two gas mixtures in the apparatus when the oxygen concentration was increased to the next higher value. At 90 percent oxygen all the energy liberated, as calculated from the evolved CO_2, was again found as heat. With but one CO_2 measurement and no heat measurements at 100 percent oxygen the rate of respiration at this concentration is uncertain in this experiment.

It is difficult to explain the sudden increase in respiration as shown by the CO_2 evolved at 78 percent oxygen. It is interesting to note, however, that the oxygen-nitrogen ratio (78 percent oxygen) which gave the maximum rate of respiration is the same as that in the nitrate ion. A further discussion will be presented later.

To demonstrate that a maximum does exist at 78 percent oxygen, the experiment was carefully repeated a month later, care being taken to remove completely the carbon dioxide from the culture by increasing the rate of aeration as the rate of respiration increased. The results are shown in figure 3, experiment T. Here, as in experiment S

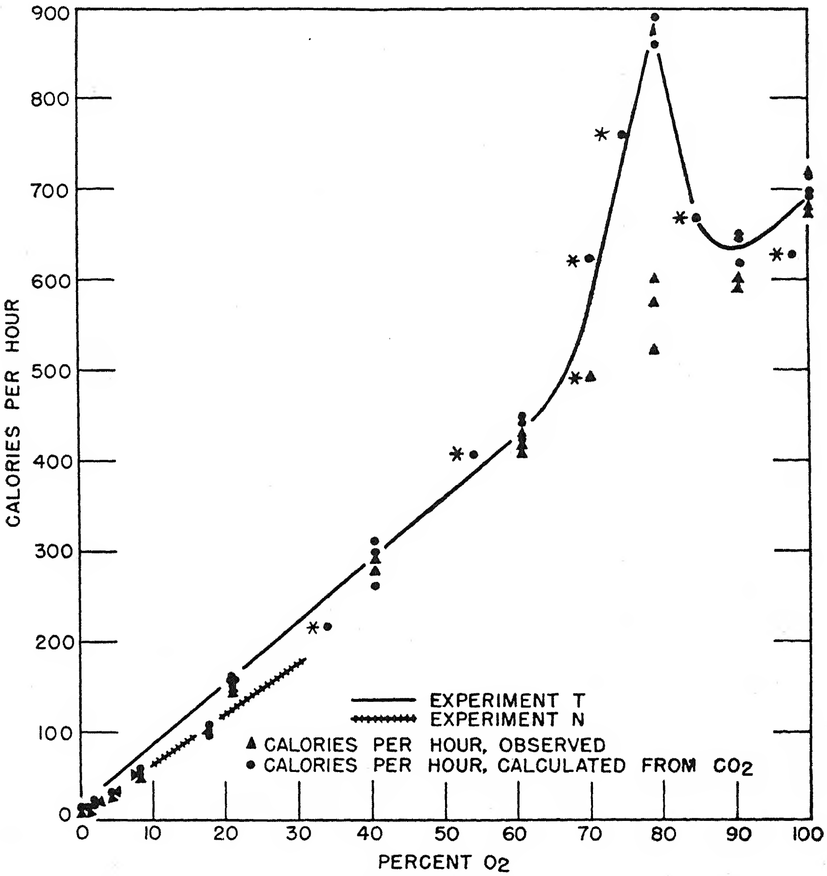

FIGURE 3.—The rate of respiration of *Azotobacter* (while fixing nitrogen) subjected to oxygen concentrations from 20 to 100 percent. The points designated by the asterisk represent the carbon dioxide evolved during 1 hour, the oxygen tension remaining at the lower value for 20 minutes and at the higher value for 40 minutes. The average oxygen tension is therefore the lower tension plus two-thirds the difference between it and the next higher oxygen tension.

(fig. 2), a maximum calculated rate of heat production occurred at 78 percent oxygen. This experiment also shows that the rate of respiration at 100 percent oxygen is slightly greater than at 90 percent. Experiment N is shown (fig. 3) in order that the slopes of the two curves may be compared.

An experiment was conducted in order to make certain that the culture was fixing nitrogen throughout the entire time the experiments were being conducted. One liter of sterile medium was transferred to a Dewar flask which was submerged in a water bath at 25° C. The

nitrogen-free medium was inoculated with 10 cc. of inoculum from the same source as that used in the other experiments. The culture was vigorously aerated with preconditioned air in a manner similar to that described earlier (*7*).

An indication of the heat produced by the culture was obtained by measuring the rise in temperature inside the Dewar flask with the aid of a Beckmann thermometer. The temperature of the water bath, in which the culture was submerged, was increased at the same rate as the temperature increased inside the Dewar flask. By this means the heat leak of the culture was reduced to a negligible amount. The amount of carbon dioxide evolved and the rise in temperature were

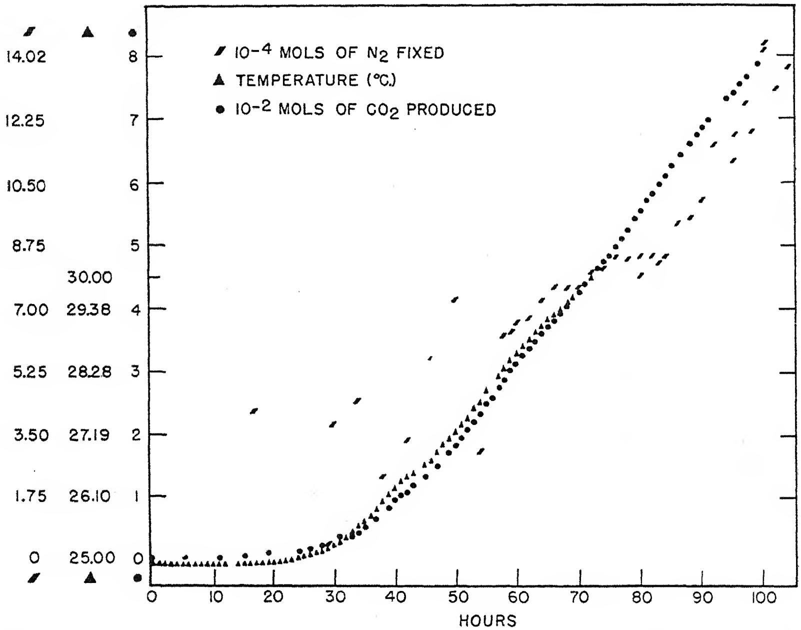

FIGURE 4.—The rise in temperature, the amount of nitrogen fixed, and the CO_2 evolved by a culture of *Azotobacter* over a period of 100 hours.

determined at regular intervals. Samples of the culture were removed at intervals and the total nitrogen determined by a standard method. The results are shown in figure 4.

It is evident that nitrogen fixation took place throughout the entire 100-hour period. It appears that after the rate of carbon dioxide production reached a constant value, the rate of nitrogen fixation also appeared to be constant and was closely correlated with the rise in temperature and the rate at which carbon dioxide was liberated.

Concerning the maximum respiration rate at 0.8 atmosphere obtained by the author (figs. 2 and 3), Lineweaver, Burk, and Horner (*11, p. 503*) state:

> * * * The value of maximum respiration at 0.8 atmosphere O_2 reported by Fife * * *[4] was obtained under conditions where oxygen pressure was not the only variable. The sugar concentration decreased considerably (about 10

[4] In Science 73:533. 1931.

gm. per liter) during the course of the experiment, and the rate of gas flow was increased at the higher oxygen pressures (private communication). The ratio of gas-liquid interface to volume of solution (as distinguished from the far less important ratio of volume of gas to volume of liquid) was critically low and any resulting lessened removal of CO_2 or establishing of respective equilibrium concentrations of O_2 (at different O_2 pressures) in all local parts of the culture medium would result in a *raised* maximum, not a lowered maximum as Fife believed.

This criticism, in the opinion of the writer, is not only in error, but is also unwarranted for several reasons. (1) the oxygen pressure was not a variable. In all experiments, the gas mixtures were supplied from tanks as described (*7*). It is obvious that the oxygen pressure remained constant at all partial pressures of oxygen studied. (2) The sugar concentration did not decrease about 10 gm. per liter as stated. The total amount of sugar present was only 10 gm. In experiment S (fig. 2), in which the maximum amount of sugar was consumed, only 50 percent was liberated as carbon dioxide. In the experiments in which combined nitrogen was supplied to the culture, only 24 percent of the available sugar was consumed. It seems logical from the mass law that such a decrease in this reactant would result in a decrease in the rate of respiration if the critical concentration was approached. (3) It is true that the rate of aeration was increased at the higher partial pressures of oxygen (*7, fig. 8*). However, a 67 percent increase in the rate of aeration above 14 liters per hour did not increase the rate of respiration. It appears that Burk and associates failed to appreciate the enormous gas-liquid interface supplied by rapid aeration with small bubbles of gas which rise through the liquid to the surface of the culture. A detailed discussion of this enormous gas-liquid interface available by rapid aeration is found elsewhere (see *7, p.* 235). It may be stated, however, that at the maximum rate of respiration (1,160 calories per hour) 231.5 cc. of oxygen was utilized by the 1,200 cc. of medium, which was only 1.5 percent of the total oxygen available. At this rate of respiration each cubic centimeter of medium absorbed 0.195 cc. of oxygen per hour. The total gas-liquid interface exposed to the culture over a period of 1 hour was 210,000 cm.^{2}; that is to say, each cubic centimeter of medium had 175 cm.^{2} of gas-liquid interface from which to absorb 0.195 cc. of oxygen in 1 hour. This does not include the interface at the surface of the medium. (4) Equilibrium was established within a few minutes upon changing the partial pressure of oxygen to either higher or lower values. This has been demonstrated (*7, fig. 6.*)

While Assimilating the Ammonium Ion

To determine whether or not the sudden increase in respiration, as measured by CO_2 produced at 78 percent oxygen, is associated with nitrogen fixation, experiments were carried out in which the culture was supplied with combined nitrogen and the rate of respiration was studied over the entire range of oxygen pressures as before. In these experiments the ammonium ion was added to prevent fixation of atmospheric nitrogen. The medium was the same as that previously used, except that 200 mg. of nitrogen as ammonium sulfate was added. The medium was inoculated and aerated as before and then transferred aseptically to the sterile calorimeter. Aeration was at once begun with 20 percent oxygen and continued until the rate of heat and CO_2 production became constant.

The experiment was then begun by aerating with 0.1 percent oxygen at the rate of 15.7 liters per hour. One percent oxygen was next added, and from then on the oxygen concentrations were increased until the culture was subjected to pure oxygen. As the rate of CO_2 production increased, the gas-liquid interface was increased by increasing the rate of aeration, in order to maintain the CO_2 concentration in the medium at a minimum. The air flow was increased from 15.7 to 17.5 liters per hour when 40 percent oxygen was added, and at 60 percent oxygen it was increased to 18 liters per hour. When 80 and 100 percent oxygen was added, the rate of air flow was increased to 19.5 liters per hour. The effect of different oxygen con-

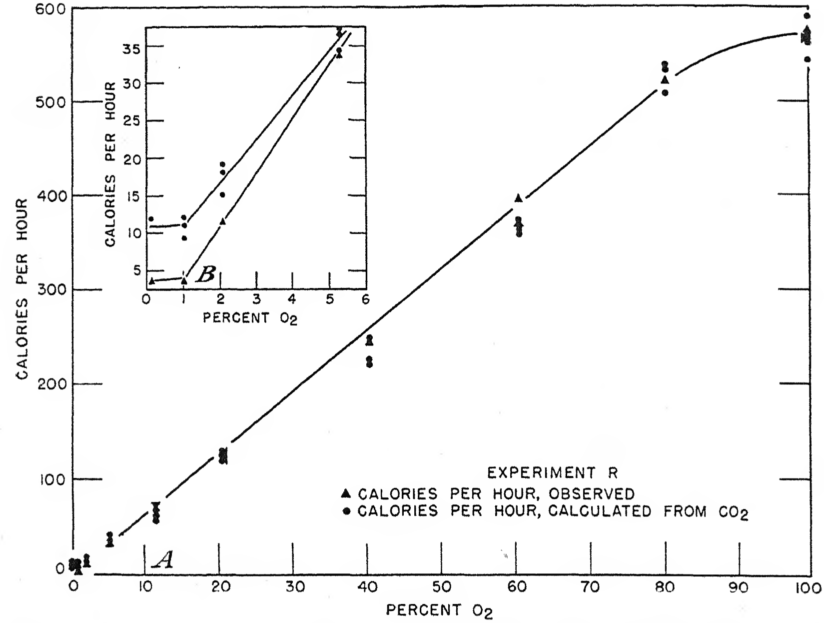

FIGURE 5.—*A*, The rate of respiration of *Azotobacter* (while assimilating the ammonium ion) under oxygen concentrations from 0.1 to 100 percent; *B*, data for low oxygen concentrations plotted on an enlarged scale.

centrations on the rate of respiration, when the ammonium ion was assimilated, is summarized in figure 5.

Apparently the rate of air flow was insufficient at 40 percent to remove the carbon dioxide, in spite of the increased rate of air flow. The increase made at 60 percent improved conditions, but not until the air flow was increased to 19.5 liters per hour at 80 percent oxygen was it sufficient to remove the carbon dioxide effectively. Here the rate of respiration is almost directly proportional to the partial pressure of oxygen between 2 and 80 percent. From 80 to 100 percent oxygen, the rate of respiration continued to increase but at a slower rate. It will be noticed that the curve also flattens out below 2 percent oxygen (*fig. 5, B*), thus showing that at these low concentrations the rate of respiration is not affected by a further decrease in

the partial pressure of oxygen. The curve also shows the extent to which intramolecular respiration is taking place.

Column 4 of table 2 shows that the respiratory quotient of *Azotobacter* did not reach unity until an oxygen concentration of 5 percent was reached. It appears that *Azotobacter* is able to catalyze fermentation reactions to a considerable extent at the lower oxygen pressures when the ammonium ion is present and even to a small degree in concentrations of oxygen as high as 5 percent. No sudden increase in the rate of respiration at 78 percent oxygen was obtained when combined nitrogen in the form of the ammonium ion was supplied to the culture.

TABLE 2.—*Respiratory quotient of a culture of Azotobacter while assimilating the ammonium ion under different partial pressures of oxygen*

Oxygen (percent)	Average energy values per hour		Respiratory quotient (CO_2/O_2)	Oxygen (percent)	Average energy values per hour		Respiratory quotient (CO_2/O_2)
	Calculated from CO_2 evolved	Observed			Calculated from CO_2 evolved	Observed	
	Calories	*Calories*			*Calories*	*Calories*	
0.1	12.4	3.5	3.54	20.4	123	120	1.01
1.0	10.7	3.5	3.09	40.3	228	244	.94
2.1	16.8	11.5	1.46	60.8	364	370	.98
5.3	38.8	33.5	1.16	80.7	521	522	1.00
11.5	63.3	71.6	.89	99.9	566	575	.98

WHILE ASSIMILATING THE NITRATE ION

To study the rate of respiration of a culture of *Azotobacter* while in the process of assimilating the nitrate ion, the above experiment was carefully repeated except that the medium contained 200 mg. of nitrate nitrogen instead of the ammonium ion. This medium had a pH of 7.1 as compared to 7.2 for the ammonia experiment. The rate of air flow was 14 liters per hour for the lower oxygen concentrations. At 40 percent oxygen the air flow was increased to 15.7 liters per hour; at 100 percent, to 17.8 liters. This experiment is summarized in figure 6.

From 10 percent to pure oxygen the rate of respiration was directly proportional to the partial pressure of oxygen, as measured both by the heat produced and the carbon dioxide evolved. Owing to the clogging of the cooling coil inside the calorimeter it was impossible to make heat measurements and thereby confirm the carbon dioxide measurements at 100 percent oxygen.

It is quite evident from fig. 6, *B*, and table 3 that anaerobic dissimilation and probably nitrate reduction takes place to a large extent below 5 percent oxygen. The respiratory quotient decreased from 5 at 1 percent to 0.97 at 11.5 percent oxygen. It is evident that the high respiratory quotients cannot be ascribed to a lack of atmospheric oxygen, since less than 1 percent of the available oxygen was utilized. The fact that the rate of respiration of *Azotobacter* was greater in the presence of the nitrate ion than when the ammonium ion was available indicates that the nitrate ion may be preferred.

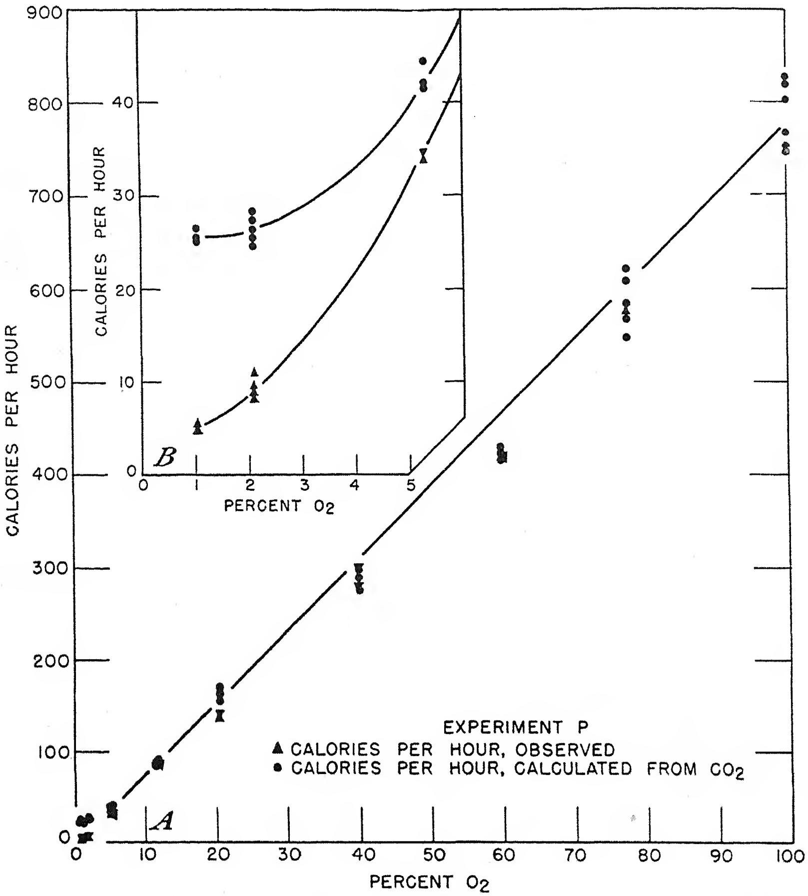

FIGURE 6.—*A*, the rate of respiration of *Azotobacter* (while assimilating the nitrate ion) under oxygen concentrations ranging from 1 to 100 percent; *B*, the data for low oxygen concentrations plotted on an enlarged scale.

TABLE 3.—*Respiratory quotients of Azotobacter obtained while the culture was assimilating the nitrate ion under increasing partial pressures of oxygen*

Oxygen (percent)	Average energy values per hour		Respiratory quotient (CO_2/O_2)	Oxygen (percent)	Average energy values per hour		Respiratory quotient (CO_2/O_2)
	Calculated from CO_2 evolved	Observed			Calculated from CO_2 evolved	Observed	
	Calories	*Calories*			*Calories*	*Calories*	
1.0	25.3	5.1	5.0	40.0	280	290	0.97
2.1	26.4	8.8	3.0	60.0	422	420	1.00
5.3	42.7	33.5	1.3	77.2	598	580	1.03
11.5	87.2	90.0	.97	99.9	833		
20.4	156	145	1.07				

CHANGE IN THE RESPIRATORY QUOTIENT WITH DECREASING PARTIAL PRESSURES OF OXYGEN

Since the publication of the free-energy data by Lewis and Randall (*9*), attempts have been made to determine the efficiency of nitrogen fixation by *Azotobacter* at different oxygen concentrations. It must be remembered that the free energy liberated by intramolecular reactions is as available to the cell as that liberated by the consumption of free oxygen, provided the reaction releasing such free energy is a step in the series of reactions which are vital to the cell. In view of this, the

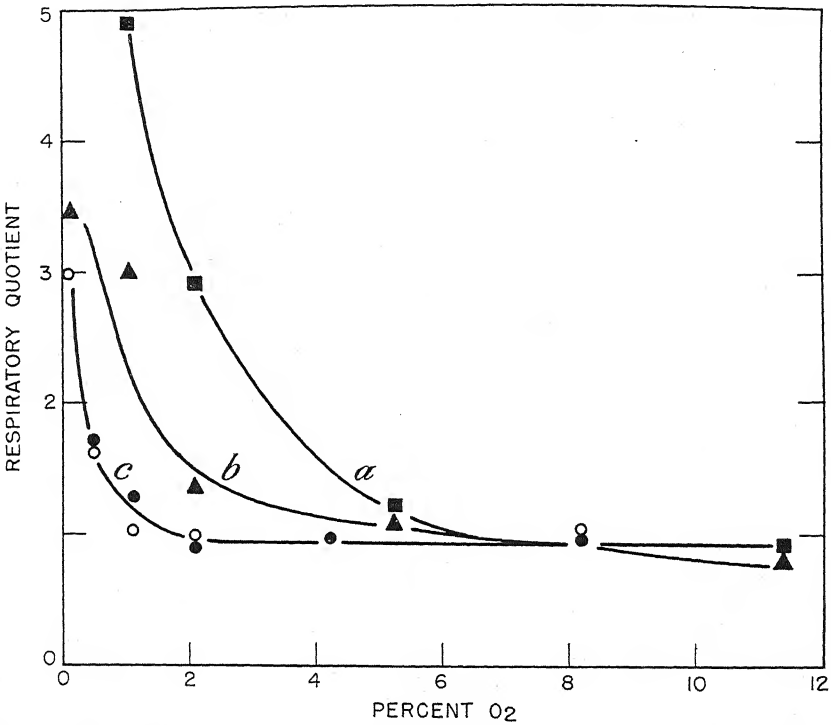

FIGURE 7.—The increase in intramolecular respiration with decreasing concentrations of oxygen by *Azotobacter* during nitrogen fixation and nitrogen assimilation: *a*, Nitrate-ion assimilation; *b*, ammonium-ion assimilation; *c*, nitrogen fixation.

energy liberated by intramolecular reactions must be taken into account before the efficiency of any cell process can be determined.

The data in figure 7 are presented to show how the respiratory quotient of *Azotobacter* increases during nitrogen fixation and nitrogen assimilation with decreasing oxygen concentrations.

It is evident from figure 7, *a* and *b*, that anaerobic dissimilation (reactions involving combined oxygen) is taking place to a marked degree by *Azotobacter* while assimilating either the nitrate or the ammonium ion. At partial pressures of oxygen greater than 5 percent, these reactions were not detectable. At this and lower values, especially in the presence of the nitrate ion, *Azotobacter* utilizes large amounts of combined oxygen with the production of carbon dioxide.

The curve clearly shows the ease with which *Azotobacter* is able to utilize combined oxygen for respiration in the presence of the nitrate ion and at low oxygen pressures where free oxygen is not readily available.

When *Azotobacter* was forced to fix atmospheric nitrogen (fig. 7, *c*) it is evident that a lower partial pressure of oxygen was necessary to force the cells to catalyze intramolecular reactions than when combined nitrogen was present. In two separate experiments the respiratory quotient was found to remain at unity until the partial pressure of oxygen was reduced to 2 percent. Below this concentration, however, the respiratory quotient increased rapidly until at 0.1 percent oxygen, where it reached a value of 3. It is clearly evident that the free oxygen consumed by *Azotobacter* at partial pressures below 2 percent is by no means a measure of the total energy liberated.

DISCUSSION

The data presented here and in previous papers (*6*, *7*) are not in agreement with those of other investigators. Meyerhof and Burk (*12*) and Burk and associates (*2*, *3*, *4*), Lineweaver (*10*), and Lineweaver and associates (*11*) have presented considerable data on the physiology of *Azotobacter* with special reference to the efficiency of nitrogen fixation and efficiency of growth. In these experiments the Warburg (*13*) apparatus was used exclusively for measuring the rate of respiration (free oxygen consumed). The efficiency of nitrogen fixation and the efficiency of growth of *Azotobacter* were determined over the entire range of oxygen pressures, with the Warburg apparatus and technique (*13*). A critical discussion of this has been given by the author (*7*). Meyerhof and Burk (*12*) report that the rate of respiration increased with increasing oxygen pressure until 15 to 20 percent oxygen was reached. Above this concentration, they found that the respiration rate fell off rapidly. The experiments of these investigators show that the rate of respiration in pure oxygen is from one-third to one-half the value for air.

The author has failed to confirm the findings of these workers. In 10 experiments (examples of which are shown in figures 1, 2, 3, 5, and 6) more than 100 separate measurements on the rate of respiration of *Azotobacter* were made, in which five concentrations of oxygen greater than that in air were used. Each of these separate measurements (eight examples of which are shown in detail in figure 6 of an earlier paper) (*7*) consisted of measuring simultaneously the heat and carbon dioxide produced over a period of at least 2 hours. In every case the author found the rate of respiration of *Azotobacter* in concentrations of oxygen above 20 percent to be greater than at 20 percent.

The duration of experiments S (fig. 2) and T (fig. 3) was 38 and 36 hours, respectively. By making the heat and carbon dioxide measurements continuously for the entire time in each experiment, any decrease in the rate of respiration would surely have been detected. Continuous heat and carbon dioxide measurements were also made in experiments R (fig. 5) and P (fig. 6) which were of 80 and 140 hours duration, respectively, with no indication of a decrease in the rate of respiration at the higher oxygen concentrations.

It is difficult to see how equilibrium between the gas and liquid

phases could have been reached and maintained with the apparatus used by Meyerhof and Burk, in the light of the data presented by the author, which demonstrates the precautions necessary to supply the culture with adequate oxygen. It is doubtful whether the gas exchange could proceed rapidly enough at the higher oxygen pressures in the Warburg apparatus to allow respiration to take place at a maximum rate. In view of the fact that the total volume of their apparatus was only 12 cc., convection currents in both the gas and liquid phase would be very small. Circulation of the gas would also be reduced to a minimum owing to the shape of the apparatus and the constant temperature at which it was maintained. It is true that the whole apparatus was rocked back and forth at a constant rate. However, this rate (120 cycles per minute, according to observations made by the author) was probably not rapid enough to break the surface of the liquid and by actual measurements the liquid was raised approximately 2 mm. on the side of a vessel similar to that used by Meyerhof and Burk (*12*) when the rate of shaking was 120 cycles per minute at an amplitude of 3 cm.

A striking example of the need for vigorous stirring was clearly demonstrated in an earlier paper (*7, fig. 9*). The points designated by the asterisk were obtained while the mechanical stirrer was idle. Although the culture was being vigorously aerated at the rate of 14 liters per hour, the stirring by aeration alone was only sufficient to produce a rate of respiration one-half of the maximum. In spite of such a high rate of aeration and a large gas-liquid interface, vigorous mechanical stirring was found to be indispensable for the cells to respire at a maximum rate.

Meyerhof and Burk (*12*) apparently assumed that the amounts of oxygen consumed were too small to alter the concentration of the gas phase. By actual measurement the author found that a maximum of 0.21 cc. of oxygen was consumed per cubic centimeter of medium per hour. It may well be that in their experiments the carbon dioxide was not removed from the medium rapidly enough to insure the most rapid rate of respiration, especially at the higher oxygen concentrations. As the pH of the medium used by these investigators was 7.3, this alone would retard the carbon dioxide from diffusing from the medium into the atmosphere above, at least until the concentration of carbon dioxide in the medium became appreciable. Moreover, the concentration of carbon dioxide necessary to become toxic would be reached much sooner at the higher oxygen pressures, due to increased rate of respiration. As a consequence, the rate of respiration would probably fall off sharply.

In the experiments performed by Meyerhof and Burk the ratio of carbon dioxide-free air to the volume of the culture was about 6 to 1 and the air was not bubbled through the culture medium. The author found it difficult to remove effectively the carbon dioxide at the higher oxygen concentrations when the carbon dioxide-free air bubbled through the well mechanically stirred medium (pH 7.2). This was found to be true even when the ratio of carbon dioxide-free air to the volume of medium was 15 to 1. As a matter of fact, in some experiments this ratio had to be increased to 18 to 1 (with corresponding increase in the gas-liquid interface) before the carbon dioxide was effectively removed. The necessity of vigorous aeration for securing a maximum rate of respiration is seen clearly when it is realized that

the 18 liter per hour rate completely replaced and swept out all the gas in the calorimeter once every minute.

It appears that the Warburg apparatus as used by Meyerhof and Burk (*12*) and Burk (*2, 3*) and Burk and Lineweaver (*4*) is probably unreliable and incapable of effecting the very rapid and large gas exchange that is necessary if *Azotobacter* is to respire at a maximum rate at the higher oxygen pressures. Hunter (*8*) found that the maximum growth rate for *Azotobacter* could be obtained only under conditions of vigorous aeration.

Meyerhof and Burk (*12*) and Burk (*3*) concluded from certain of their experiments that the nitrogen fixation process is more efficient (less energy is expended per milligram of nitrogen fixed) at the lower oxygen pressures. They state that the efficiency is 10 to 20 times greater at 0.1 percent oxygen than in air. These investigators prepared the experimental organisms by first growing them for 24 to 48 hours in 250-cc. gas wash bottles containing 50 cc. of culture medium.

During this period the cultures were aerated with compressed air (21 percent oxygen) at the rate of 100 cc. per minute. After proper dilution with sterile media to obtain the desired number of organisms, 3 to 5 cc. of this culture was transferred to the respiration vessel. It is important to point out that the cells and medium have been aerated and in contact with air containing the normal amount of oxygen. Consequently, the cells and media are saturated with oxygen at a pressure equal to that in air.

In order to study the efficiency of nitrogen fixation at 0.1 percent oxygen these workers passed this gas mixture down through the manometer stopcock and out of the ground-glass opening in the neck of the side cup of the reaction vessel at the rate of 1 to 2 liters per minute for 2 or 3 minutes. The stopcock and side cup were then closed simultaneously. At the maximum these investigators relied on 6 liters of gas flowing through the apparatus to sweep all the air from such an irregular-shaped vessel. At the same time they attempted to establish a gas mixture of 0.1 percent oxygen and to reduce the oxygen concentration of the culture medium from 20 in one case to 0.12 percent oxygen in 2 to 3 minutes without the gas bubbling through the medium.

It is difficult to see how the system set up by the above investigators could be brought to a steady state with respect to the gas-medium phase, especially where a 200-fold oxygen-pressure change was involved, with only 6 liters of gas passing over the medium in the course of 2 to 3 minutes. The data presented by the author (*7, fig. 7*) show that it required from 8 to 14 hours for no less than 160 liters of gas to bubble through the well-stirred medium. It appears from the data presented here that the experiments of Meyerhof and Burk on the efficiency of nitrogen fixation were completed before equilibrium was reached at the lower partial pressures of oxygen.

It must be remembered that it is not the partial pressure of oxygen over or in the culture medium that ultimately controls the rate of respiration, but the partial pressure of oxygen within the living cells where the reaction is taking place. It is the concentration of oxygen at this point that must be reduced from 20 to 0.1 percent before it can be said that the organisms are respiring under the influence of the lower gas mixture.

Meyerhof and Burk (*12, p. 139*) present a curve the data for which

were obtained by the above-described technique. The data show an increase in efficiency of nitrogen fixation with decreasing oxygen pressures. These investigators point out from this curve that the efficiency of nitrogen fixation increases from approximately 1 percent at 0.20 atmosphere to approximately 4 percent at 0.02 atmosphere of oxygen. At this point the slope of the curve is shown to increase rapidly as the partial pressure of oxygen approaches zero. Under these same conditions, the efficiency of nitrogen fixation is shown to approach a value of 12 percent.

When studying the energy relations of a biological process at the lower partial pressures of oxygen, where intramolecular respiration is possible, a measure of only the free oxygen consumed would be unreliable and misleading. If an organism is catalyzing several energy-yielding reactions in which combined oxygen and free oxygen are utilized simultaneously, it is evident that a measurement of only the free oxygen consumed would be of little value in determining the total energy liberated. Similarly, if only the carbon dioxide evolved were measured in such a case, it too would fail to point out the true energy relations.

As these investigators based their entire measurements on the free oxygen absorbed, it is only natural that they should observe increased efficiencies of nitrogen fixation at the very low oxygen pressures because of intramolecular respiration. (See figure 7 for the extent to which intramolecular respiration takes place while both nitrogen fixation and nitrogen assimilation are taking place.)

The conclusions drawn by Meyerhof and Burk (*12*) on the efficiency of nitrogen fixation, and also by Burk and Lineweaver (*4*) on the efficiency of growth at the lower oxygen pressures are therefore questionable for two reasons: (1) When partial pressures of oxygen lower than 5 percent were used, true equilibrium was probably not obtained; that is, the oxygen absorbed by the medium and the cells was not completely removed and a steady state established at the lower oxygen value before the experiments were begun. No data were presented on this point. (2) Meyerhof and Burk did not attempt to measure the intramolecular respiration that took place. It must be admitted that conditions are extremely favorable at such low pressures of oxygen for the culture to catalyze such reactions and the free energy liberated by them must be considered in the efficiency of the nitrogen fixation process and the efficiency of growth.

That *Azotobacter* does catalyze reactions not involving free oxygen with the liberation of energy cannot be doubted in the light of Bonazzi's (*1*) experiments which have been well confirmed by the author. The extent to which such reactions take place is brought out in tables 1, 2, 3; in *B* of figures 1, 2, 5, and 6; and in figure 7. When the culture was forced to fix nitrogen, it is seen that at 0.1 percent oxygen only one-third of the carbon dioxide produced could be accounted for by the heat evolved. In other words, at 0.1 percent oxygen, respiration with free oxygen was but one-third of the total respiration that took place.

From table 1, it is seen that the gas mixture must contain at least 2 percent oxygen before reactions involving combined oxygen are reduced to a point at which they are negligible. When *Azotobacter* was respiring in the presence of combined nitrogen, the experiments show that it required considerably higher concentrations of oxygen to

reduce intramolecular respiration to a minimum. It is evident that the amount of energy liberated by intramolecular reactions is appreciable, in proportion to the total amount liberated, at the low oxygen pressures.

There are several points of striking interest in the data presented which may shed some light on the mechanism of nitrogen fixation. It was shown in a previous paper (*6*) that the sudden increase in the rate of respiration during nitrogen fixation, as measured by the carbon dioxide evolved, occurred at the same oxygen-nitrogen ratio (78 percent oxygen) as found in the nitrate ion. At this oxygen value (figs. 2 and 3) the heat produced was only 62 and 52 percent of that calculated from the carbon dioxide evolved for the two experiments reported. That this fact is significant is evident when it is recalled that no maximum rate of respiration as measured by the carbon dioxide evolved occurred at this oxygen tension when nitrogen in the form of the ammonium or the nitrate ion was supplied to the culture.

In every case in which nitrogen in a combined form was supplied to the culture the heat measured was equal to that calculated from the carbon dioxide evolved in the regions where intramolecular respiration was absent.

It is interesting to note that the increase in rate of respiration per unit increase in the partial pressure of oxygen is greatest when the culture is assimilating the nitrate ion. Here the rate of respiration is directly proportional to the partial pressure of oxygen. This ratio of unity remained constant over the entire oxygen range above 5 percent. It required a greater concentration of oxygen to prevent intramolecular respiration when the culture was assimilating the nitrate ion than when nitrogen was supplied as an ammonium salt or by nitrogen fixation.

Bonazzi (*1*), on recalculation of data published by Hills, pointed out that *Azotobacter* is able to liberate nitrogen by utilizing the oxygen from the nitrate ion and that the nitrate ion is used by *Azotobacter* in preference to the ammonium ion.

The data presented here show that the metabolic activities of *Azotobacter* are greater in the presence of the nitrate ion than in the presence of the ammonium ion. This would also indicate a preference for the nitrate ion by *Azotobacter*.

If Bonazzi's interpretation of data published by Hills is correct, it would appear that *Azotobacter* is capable of catalyzing the reaction $2NO_3 \rightarrow N_2 + 3O_2$.

The fact that below 5.0 percent oxygen *Azotobacter* catalyzes certain reactions not involving free oxygen (this is shown by the fact that the heat evolved is much less than that calculated from the carbon dioxide produced (fig. 6)) may also indicate that oxygen is being taken from the nitrate ion. If the oxygen is not taken from the nitrate ion then the presence of the nitrate ion is unique in that it is instrumental in causing the oxygen to be removed from other compounds and liberated as carbon dioxide. From these considerations it is not unreasonable to suppose that *Azotobacter* may catalyze the above reaction in either direction.

Several writers have suggested that the first product of nitrogen fixation by *Azotobacter* is ammonia. There is, however, no conclusive

evidence to support this contention. Burk and Horner *(5 p. 121)* state that—

> the mere occurrence of ammonia in cultures of *Azotobacter* grown in N_2 cannot be regarded as critical evidence in favor of a view current that the ammonia observed is derived, either wholly or in any part, specifically and directly from N_2.

It is true that it is the reduced form of nitrogen which the organisms ultimately use to build protoplasm. It must be remembered, however, that the first product of nitrogen fixation will depend entirely on the catalysts which the organism has at its disposal and not on the form of nitrogen from which is built the protein complex of the cells.

Burk (*3*) discussed the mechanism of respiration of *Azotobacter* in the light of his data and from the point of view of chain kinetics and contact catalysis. He concluded that both oxygen and the glucose molecule must be in direct contact with the solid respiration catalyst if the reaction is to take place measurably, and that bombardments by unadsorbed molecules were ineffective.

From the fact that the rate of respiration of *Azotobacter* continues to increase with increasing partial pressures of oxygen, as shown by the data presented in this paper, it is clear that oxygen up to 1 atmosphere pressure does not inhibit the rate of its own consumption. It is evident from the data presented that the rate of respiration does not violate the mass law, as stated by Burk (*3*, *p. 1206*). Recalling that the rate of respiration is proportional to the partial pressure of oxygen, it appears that the reaction proceeds by bombardment and that direct contact of the reactants with a solid respiration enzyme is not imperative. It is also apparent that respiration is a first order reaction with respect to oxygen.

SUMMARY

The rate of respiration of *Azotobacter* during nitrogen fixation was studied over the entire range of oxygen pressures. The rate of respiration was found to be dependent on the partial pressure of oxygen provided the carbon dioxide is kept to a low value and the medium is kept saturated with oxygen at the partial pressure in question. Below 2 percent oxygen while fixing nitrogen *Azotobacter* respires with a respiratory quotient well above unity. At all concentrations of oxygen above 2 percent the heat measured was found to be equal to that calculated except at 78 percent. Here the heat measured was found to be approximately 57 percent of that calculated from the carbon dioxide evolved.

When *Azotobacter* was assimilating the ammonium ion the rate of respiration was found to be proportional to the concentration of oxygen from 5 to 80 percent. The heat measured was found to be equal, at all concentrations of oxygen above 5 percent, to that calculated from the carbon dioxide evolved. Below 5 percent intramolecular respiration was found to increase as the partial pressure of oxygen was decreased until at 0.1 percent oxygen the respiratory quotient reached 3.54.

The rate of respiration was found to be directly proportional to the concentration of oxygen from 10 to 100 percent when *Azotobacter* was allowed to assimilate the nitrate ion for its source of nitrogen. Below 10 percent oxygen intramolecular respiration occurred to a

considerable extent. The respiratory quotient reached 5.0 at 1 percent oxygen.

When the oxygen concentration is suddenly decreased from one-twentieth to one two-hundredth of that in air, considerable time is required to reduce the partial pressure of oxygen in the culture and within the cells to a point where the rate of respiration of the culture proceeded at a minimum constant rate.

Increasing the concentration of oxygen does not decrease the rate of its own consumption under the experimental conditions to which *Azotobacter* was subjected in these studies.

The data presented indicate that the first product of nitrogen fixation may be a union of nitrogen with oxygen.

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INHERITANCE OF SEED COLOR IN LACTUCA SATIVA[1]

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INTRODUCTION

The mature fruit of lettuce is an achene or naked seed, the color of which is determined by the color of its pericarp. The pigments that give the seed its particular color seem to be localized in the pericarp tissues.[2]

The mode of inheritance of seed color is of immediate practical importance to those breeding lettuce for disease resistance, climatic adaptation, and desirable commercial characteristics. Unlike flowers of most other food crops, those of lettuce can be emasculated only with extreme difficulty, if at all. In making crosses the breeder must remove the maternal parent's own pollen from the stigmas after the anthers have dehisced, but before the pollen tubes have entered the stigmatic tissues. As it is generally almost impossible to remove every grain of the maternal parent's pollen, both selfed and hybrid seed develop in heads in which pollen removal is attempted. Therefore, the breeder must know the mode of inheritance of some easily recognizable character by which he can distinguish hybrid from selfed plants in the F_1 generation. Seed color is a character that can be used to advantage for this purpose.

The cultivated varieties of lettuce (*Lactuca sativa* L.) may be roughly divided into 3 groups on the basis of their achene color. Of the 114 varieties of lettuce listed by Tracy[3] in 1904, 81 were indicated to have white, 30 black, and 3 yellow seed. Since then the trend has been even more toward the white-seeded varieties, until now there are only a few important varieties of the black-seeded type and no important varieties in the yellow-seeded group.

In the white-seeded group, the color is fairly constant except for discolorations caused by premature harvesting, dampness, and other environmental factors. In the black-seeded group the achenes range in color from reddish brown through dark brown to black. These different shades probably have a genetic basis; however, the differences are not great enough to permit accurate classification in view of the minor color variations caused by environmental factors. There are also some shade variations among varieties in the yellow group

Some data have been published by Durst[4] and Thompson[5] on the inheritance of factors for black and white seeds, but no report that deals with the factors for yellow seed has been found. The data of Durst and Thompson agree that black behaves as a monogenic factor

[1] Received for publication September 11, 1942.

[2] Borthwick, H. A. Unpublished data on the morphology of lettuce seed.

[3] Tracy, W. W., Jr. AMERICAN VARIETIES OF LETTUCE. U. S. Bur. Plant Indus. Bul. 69, 103 pp., illus. 1904.

[4] Durst, C. E. INHERITANCE IN LETTUCE. Ill. Agr. Expt. Sta. Bul. 356, pp. [237]-341, illus. 1930.

[5] Thompson, R. C. GENETIC RELATIONS OF SOME COLOR FACTORS IN LETTUCE. U. S. Dept. Agr. Tech. Bul. 620, 38 pp., illus. 1938.

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dominant to white. It is the purpose of this paper to show the relation of the factors that control the inheritance of the three colors, but no effort was made in this study to account for the inheritance of the minor color variations within the three large groups.

MATERIALS AND METHODS

The data presented were obtained from numerous hybrid populations of lettuce stocks being grown at the Bureau of Plant Industry Station, Beltsville, Md., for breeding heat-tolerant and disease-resistant varieties. The plants were grown either in 10-inch clay pots or in 1-foot by 2-foot by 3-inch cypress planting flats in a screened greenhouse. The possibility of contamination by insects was kept at a minimum by frequent spraying and fumigation.

The parent stocks had been grown for at least two generations previous to the cross from which seed-color records were kept and were known to be homozygous for the particular color type. The parent stocks used for the black phenotype were from a strain of Grand Rapids that has been maintained by the Division of Fruit and Vegetable Crops and Diseases for many years. For the white phenotype the variety Chavigne from Vilmorin-Andrieux & Cie., Paris, France; Iceberg from the Ferry-Morse Seed Co., San Francisco, Calif.; and a hybrid selection from a breeding stock were used. The variety Giant Summer from the Ferry-Morse Seed Co. was used for the yellow-seeded parent type.

In all cases the flower heads of the maternal parent were depollinated with water and dried before the desired pollen was applied by brushing the washed stigmas with the pollen-laden styles of flowers from the male parent.

GENETIC RELATIONS OF FACTORS FOR ACHENE COLOR

WORKING HYPOTHESIS

The breeding behavior of the three color types indicates that achene color in lettuce is inherited in a manner similar to that of the factors (*CcAa*) for coat color in rodents in which an F_2 generation from a cross between a black (*CCaa*) and an albino (*ccAA*) consists of 9 agouti, 3 black, and 4 albino.

This requires the presence of an independent allelomorphic pair in addition to those previously suggested by Durst [6] and Thompson [7]. In these two reports the genetic factor for black was represented by the symbol *WW* and the white by the symbol *ww*. This new pair of allelomorphs has been given the symbol *Yy*. The symbolized genotypes and their phenotypic expressions are *WWYY*, black; *wwYY*, yellow; *WWyy*, white; and *wwyy*, white. All four of these homozygous genotypes have been isolated, and their genetic constitution has been studied.

CROSS INVOLVING BLACK- AND YELLOW-SEEDED PARENTS

Grand Rapids × *Giant Summer* (*cross No. 122*).—Pollen from flowers of the yellow-seeded variety Giant Summer (*wwYY*) was applied to washed stigmas of flowers of the black-seeded variety Grand Rapids (*WWYY*). The cross was made in this way so that it might be pos-

[6] See footnote 4.
[7] See footnote 5.

sible to isolate at an early stage the hybrids from the selfed plants in the resulting progenies. Such isolation is possible because, as Thompson has shown,[8] the spotted anthocyanin leaf type of Giant Summer is dominant to the absence of pigment in the leaves of Grand Rapids.

A population of 93 F_2 plants was grown, with a ratio of 73 black-seeded to 20 yellow-seeded resulting. This is a satisfactory fit to a 3 to 1 ratio.

F_3 progenies were grown from both black- and yellow-seeded F_2 plants. Progenies from the F_2 yellow-seeded plants were all yellow-seeded. In the progenies from the black-seeded F_2 plants there were some families that were all black-seeded and other families with black- and yellow-seeded plants in the ratio of approximately 3 black-seeded to 1 yellow-seeded. The results from the F_2 and F_3 generations are given in tables 1 and 2, respectively.

TABLE 1.—*Records for F_2 progenies from selfed F_1 plants from crosses involving black-, yellow-, and white-seeded varieties of lettuce*

Phenotypes and cross	Parental varieties and assumed genotypes	F_1 plants	F_1 seed color	F_2 progenies				Assumed ratio	χ^2
				Black	Yellow	White	Total		
Black-seeded × yellow-seeded:									
No. 122	Grand Rapids (*WWYY*) × Giant Summer (*wwYY*).	*Number* 17	Black	*Number* 73	*Number* 20	*Number* 0	*Number* 93	3:1	[1]0.5198
Black-seeded × white-seeded:									
No. 12	Grand Rapids (*WWYY*) × Iceberg (*WWyy*).	27	do	89	0	19	108	3:1	4.0000
No. 396	Grand Rapids (*WWYY*) × hybrid (*wwyy*).	35	do	88	42	52	182	9:3:4	[2]4.5866
White-seeded × yellow-seeded:									
No. 21	Chavigne (*WWyy*) × Giant Summer (*wwYY*).	42	do	96	36	46	178	9:3:4	.4549
No. 40	Hybrid (*wwyy*) × Giant Summer (*wwYY*).	28	Yellow	0	42	13	55	3:1	.0958

[1] χ^2 for 1 degree of freedom (3:1 ratio) at 1-percent level, 6.635; at 5-percent level, 3.841.
[2] χ^2 for 2 degrees of freedom (9:3:4 ratio) at 1-percent level, 9.210; at 5-percent level, 5.991.

CROSSES INVOLVING BLACK- AND WHITE-SEEDED PARENTS

Grand Rapids × Iceberg (cross No. 12).—The white-seeded variety Iceberg (*WWyy*) was used as the pollen parent in a cross with the black-seeded Grand Rapids (*WWYY*). All the 27 F_1 plants grown to maturity produced black seed. The F_2 progenies segregated in the ratio of approximately 3 black- to 1 white-seeded. In the F_3 generation all the white-seeded F_2 plants gave families that produced only white seed. The black-seeded F_2 plants gave some families that were all black-seeded and other families that were black- and white-seeded in the ratio of approximately 3 black to 1 white. The F_2 and F_3 data are given in tables 1 and 2, respectively.

Grand Rapids × white-seeded hybrid (cross No. 396).—The white-seeded parent (*wwyy*) used in this cross was isolated from an F_3

[8] See footnote 5.

TABLE 2.—*Records for F_3 progenies from selfed F_2 plants from crosses involving black-, yellow-, and white-seeded varieties of lettuce*

Phenotypes and cross	Parental varieties and assumed genotypes	F_2 families	F_2 seed color	F_3 progenies: Black	F_3 progenies: Yellow	F_3 progenies: White	F_3 progenies: Total	Assumed ratio	χ^2
		Number		*Number*	*Number*	*Number*	*Number*		
Black-seeded × yellow-seeded:									
No. 122	Grand Rapids (*WWYY*)×Giant Summer (*wwYY*)	6	Black	49	0	0	49		
		11	do	62	15	0	77	3:1	[1] 1.1409
		3	Yellow	0	31	0	31		
Black-seeded × white-seeded:									
No. 12	Grand Rapids (*WWYY*) × Iceberg (*WWyy*).	9	Black	51	0	0	51		
		15	do	75	0	20	95	3:1	.7895
		4	White	0	0	44	44		
No. 396	Grand Rapids (*WWYY* × hybrid (*wwyy*).	9	Black	87	0	0	87		
		10	do	71	28	0	99	3:1	.5690
		15	do	111	0	30	141	3:1	1.0425
		37	do	197	71	96	364	9:3:4	[2] .6788
		17	Yellow	0	127	36	163	3:1	.7383
		5	do	0	47	0	47		
		27	White	0	0	259	259		
White-seeded × yellow-seeded:									
No. 21	Chavigne (*WWyy*) × Giant Summer (*wwYY*).	6	Black	56	0	0	56		
		18	do	126	47	0	173	3:1	.4952
		13	do	98	0	29	127	3:1	.3760
		29	do	151	50	80	281	9:3:4	2.0405
		11	Yellow	0	91	17	108	3:1	4.9382
		8	do	0	77	0	77		
		35	White	0	0	341	341		
No. 40	Hybrid (*wwyy*) × Giant Summer (*wwYY*).	3	Yellow	0	29	0	29		
		12	do	0	70	17	87	3:1	1.5209
		5	White	0	0	35	35		

[1] χ^2 for 1 degree of freedom (3:1 ratio) at 1-percent level, 6.635; at 5-percent level, 3.841.
[2] χ^2 for 2 degrees of freedom (9:3:4 ratio) at 1-percent level, 9.210; at 5-percent level, 5.991.

population of the cross No. 21 between the white-seeded Chavigne and the yellow-seeded Giant Summer. As indicated in the discussion of cross No. 21, one-fourth of the F_2 plants produced white seed. According to the hypothesis, one-fourth of these F_2 white-seeded plants should be of the double-recessive (*wwyy*) genotype. Seven of these F_2 white-seeded segregates were tested by backcrossing to the yellow-seeded Giant Summer, and one of these was found to be of the double-recessive genotype, as indicated by an all yellow-seeded F_1 generation and an F_2 generation that gave 3 yellow-seeded to 1 white-seeded. This double recessive was then crossed with Grand Rapids (*WWYY*). The F_1 plants of this cross were black-seeded, and the F_2 segregated approximately 9 black-seeded, 3 yellow-seeded, and 4 white-seeded.

The F_3 progenies from the white-seeded F_2 plants were all white-seeded. Some progenies from the black-seeded F_2 plants produced only the black-seeded type; some produced 3 black-seeded to 1 yellow-seeded; some 9 black-seeded, 3 yellow-seeded, and 4 white-seeded; and some 3 black-seeded to 1 white-seeded. Some progenies of yellow-seeded F_2 plants produced all yellow-seeded F_3 and others 3 yellow-seeded to 1 white-seeded. The F_2 and F_3 data are given in tables 1 and 2, respectively.

CROSSES INVOLVING YELLOW- AND WHITE-SEEDED PARENTS

Chavigne × Giant Summer (cross No. 21).—The yellow-seeded variety Giant Summer (*wwYY*) was used as the pollen parent in a cross with the white-seeded variety Chavigne (*WWyy*). The F_1

hybrid plants all produced black seed. The F_2 population segregated 9 black-seeded, 3 yellow-seeded, and 4 white-seeded. The F_3 progenies from white-seeded F_2 plants gave only white seed. The F_3 progenies from yellow-seeded F_2 plants gave some families that were all yellow-seeded and other families that segregated with a moderately significant departure from 3 yellow- to 1 white-seeded. F_3 progenies from black-seeded F_2 plants were all black-seeded, 3 black-seeded to 1 yellow-seeded, 3 black-seeded to 1 white-seeded, or 9 black-seeded to 3 yellow-seeded to 4 white-seeded. The F_2 and F_3 data are presented in tables 1 and 2, respectively.

White-seeded hybrid × Giant Summer (cross No. 40).—The yellow-seeded Giant Summer (*wwYY*) was used as the pollen parent in a backcross to the white-seeded F_3 selection (*wwyy*) from Chavigne × Giant Summer. The F_1 backcrossed plants all produced yellow seed, and the F_2 population from these segregated 3 yellow- to 1 white-seeded. The F_3 progenies from the white-seeded F_2 plants were all white-seeded. From the F_2 yellow-seeded plants some families that were all yellow-seeded and other families that segregated 3 yellow-seeded to 1 white-seeded were obtained. F_2 and F_3 data are presented in tables 1 and 2, respectively.

DISCUSSION

The 3 to 1 ratio of black to white seed in the F_2 generation, as suggested by Durst [9] and by Thompson,[10] holds only in the case of the white-seeded phenotype having the *wwYY* constitution. With the double-recessive white-seeded (*wwyy*) the F_2 from a cross with a black-seeded variety gives the ratio of 9 black-seeded, 3 yellow-seeded, to 4 white-seeded.

Many varieties of lettuce of the cultivated form (*Lactuca sativa*) have come under the writer's observation in the lettuce-breeding program of the United States Department of Agriculture, and breeding data on seed color have been recorded on many of these. None of the white-seeded varieties on which breeding behavior records are available are of the double-recessive (*wwyy*) type. This type must be very rare among fixed varieties.

SUMMARY

Data from the F_1 generation and from F_2 and F_3 progenies of crosses involving the three seed-color types in lettuce—black, yellow, and white—are presented.

The data have been analyzed for an explanation of the inheritance of the factors that control pericarp color in lettuce seed. The analysis indicates that the expression of black, yellow, and white seed color in cultivated lettuce (*Lactuca sativa*) is controlled by two pairs of allelomorphs, which have been assigned the symbols *Ww* and *Yy*. The inheritance is similar to that of the factors (*CcAa*) for coat color in rodents in which the F_2 from a cross between black and albino parents gives 9 agouti, 3 black, and 4 albino. In the present case the F_2 progenies from a cross between a white-seeded (*WWyy*) and the yellow-seeded (*wwYY*) segregated 9 black-seeded, 3 yellow-seeded, and 4 white-

[9] See footnote 4.
[10] See footnote 5.

seeded. This gives one true-breeding black genotype (*WWYY*), one true-breeding yellow genotype (*wwYY*), and two true-breeding white genotypes (*WWyy*) and (*wwyy*).

The double-recessive white-seeded genotype (*wwyy*) is rare, if present at all, among commercial varieties of lettuce. A homozygous line of this genotype was obtained only by the use of testers on the white-seeded segregates from a yellow-seeded by a white-seeded cross in which the white-seeded was of the *WWyy* genotype.

INHERITANCE OF OIL GLANDS IN PIMA COTTON [1]

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INTRODUCTION

Small spherical oil glands occur in cotton, on the herbage, flowers, and bolls, usually more or less embedded in the surrounding tissue. Such glands are apparently universal in the genus *Gossypium* L., according to Watt [2] and other authors. Some data pertaining to the inheritance of oil glands in Pima cotton, an agronomic variety of *G. barbadense* L., are presented in the following account. Although taxonomists have utilized differences in characteristics of oil glands, the writers are not aware of any previous genetic study of these bodies in the genus *Gossypium*.

Smith [3] applied the designation "P Hope" to a family of Pima cotton that is characterized by smooth bolls. Smith found that "pitted" and "smooth" are inherited in monohybrid fashion, and for these characters proposed the symbols B^p and B^s, respectively. Family P Hope also has three other distinguishing characters, namely, delayed development of oil glands on the young bolls, smaller and more deeply embedded glands on the stem, and smaller glands on the calyx. The characters that distinguish P Hope from ordinary Pima cotton are described in the following section.

DESCRIPTION OF CHARACTERS

DEVELOPMENT OF BOLL GLANDS

In family P Hope, the oil glands are wanting, or at least not discernible, until the third day after anthesis. The character is termed "late." In PH8 and other families of the Pima variety that have pitted bolls, the normal condition in *Gossypium barbadense*,[4] the boll glands are "early," that is, conspicuously evident on the day following anthesis. In fact, pigmented oil glands occur on the ovary of PH8 flower buds. The aspect of bolls of P Hope, PH8, and PH8 × P Hope F_1, on the first day after anthesis, is illustrated in figure 1.

STEM GLANDS

Oil glands on the stem, or main axis, of P Hope differ in size from corresponding glands of family PH8. Although stem glands are actually as numerous in P Hope as in PH8 (table 1), the glands are deeply embedded and consequently less conspicuous.

[1] Received for publication November 13, 1942.

[2] Watt, Sir George. THE WILD AND CULTIVATED COTTON PLANTS OF THE WORLD. 406 pp., illus. London. 1907.

[3] Smith, E. G. INHERITANCE OF SMOOTH AND PITTED BOLLS IN PIMA COTTON. Jour. Agr. Res. 64: 101-103, illus. 1942.

[4] Early development of the boll glands is apparently the rule also in upland cotton (*Gossypium hirsutum* L.), in which the bolls are not pitted. Glandless bolls sometimes occur in Hopi cotton (*G. hopi* Lewton) and in some segregates of crosses involving Hopi cotton, but in these cases the glandless condition persists through maturity of the bolls.

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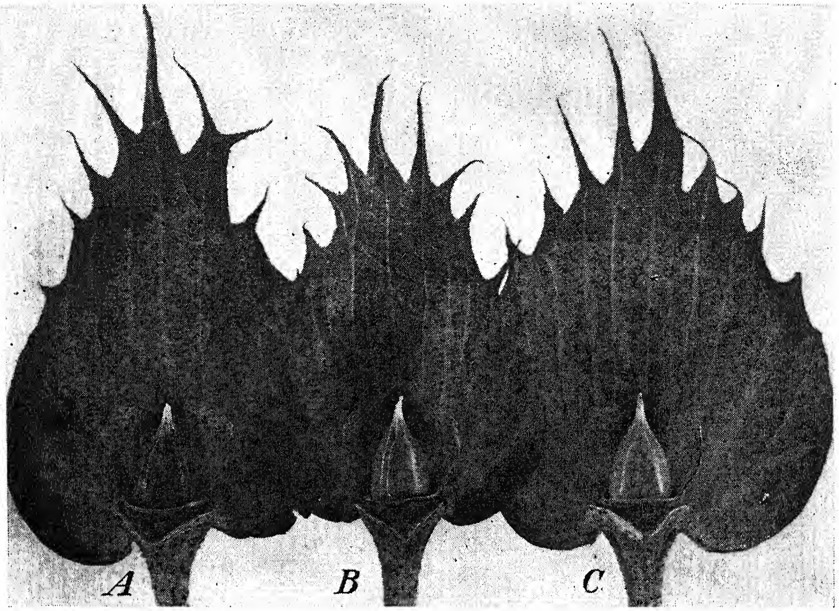

FIGURE 1.—Aspect of bolls on the first day after anthesis. The glands are numerous and conspicuous in (*A*) family PH8, comparatively sparse in (*B*) PH8 × P Hope F_1, and wanting, or at least not discernible, in (*C*) family P Hope. ×1.4.

CALYX GLANDS

The calyx glands of family P Hope, like the stem glands, are smaller than the corresponding glands of family PH8. The difference in size, although not large, is sufficient to make feasible the classification of nearly all plants in segregating populations. The relative size of the oil glands in PH8, P Hope, and PH8 × P Hope F_1 is observable in the calyxes shown in figure 1. Calyx glands are also illustrated, at greater magnification, in figure 2.

Calyx and stem glands are classified as of large, intermediate, or small size.

BOLL SURFACE

Pitted boll surface is the condition found in normal Pima cotton. The surface is dotted with conspicuous pits, and many of the oil glands are large and shallowly embedded. In family P Hope, the bolls may be described as "smooth," the surface being obscurely pitted and the oil glands small and deeply embedded (fig. 2).

As Harland [5] (*pp. 117–118*) points out, in the Peruvian group of cottons, to which the Pima variety belongs, the oil glands are near the surface, and around some of them the surface is depressed in small craterlike pits. Pits do not occur when the glands are deeply embedded, as in the Pima family P Hope and in upland cotton (*Gossypium hirsutum* L.). Therefore, it can be seen that a very close relation exists between the pitting of the boll surface and the depth at which the oil glands are embedded in the tissue.

[5] HARLAND, S. C. THE GENETICS OF COTTON. 193 pp., illus. London. 1939.

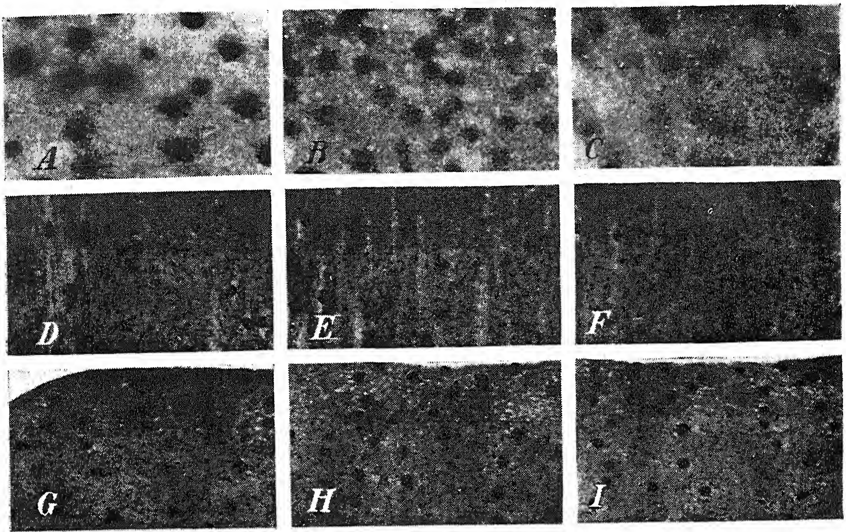

FIGURE 2.—Oil glands of bolls, stems, and calyxes in Pima cotton. Boll glands in (*A*) family PH8, (*B*) PH8 × P Hope F_1, and (*C*) family P Hope; stem glands in (*D*) PH8, (*E*) PH8 × P Hope F_1, and (*F*) P Hope; calyx glands in (*G*) PH8, (*H*) PH8 × P Hope F_1, and (*I*) P Hope. ×11.

TABLE 1.—*Classification of populations grown in 1941 in respect to character o boll surface and the mean number of oil glands in an area of 25 sq. mm. on the bolls and stems*

Population designation	Classification in respect to boll surface[1]	Number of plants	Number of oil glands per unit area			
			On bolls		On stems	
			Mean	Standard error of mean	Mean	Standard error of mean
PH8	Pitted	13	25.1	0.67	33.4	2.11
P Hope	Smooth	18	22.3	1.06	35.8	1.63
PH8 × P Hope F_1	Intermediate	17	46.4	1.23	35.9	1.50
PH8 × P Hope F_2	Pitted	10	31.2	1.62	40.8	1.34
Do.	Intermediate	20	43.3	1.19	34.7	1.49
Do.	Smooth	6	20.2	2.14	34.3	3.25
(PH8 × P Hope F_1) × PH8 F_1	Pitted	20	28.7	.78	39.4	1.54
Do.	Intermediate	21	45.9	1.23	37.0	1.72
(PH8 × P Hope F_1) × P Hope F_1	Smooth	6	23.7	2.11	37.5	3.55
Do.	Intermediate	14	48.3	1.84	38.6	1.90

[1] The three oil gland characters mentioned in the text do not segregate independently of boll surface. Therefore, in this column pitted boll surface and smooth boll surface also denote, respectively, early and late development of boll glands, large and small stem glands, and large and small calyx glands. Individuals intermediate for boll surface are also intermediate for the other characters.

In the characters described above, the F_1 is intermediate between the two parental types. This statement does not apply, however, to the number of boll glands, which will be discussed later

METHODS

All individuals in the several populations were classified independently for each character. Repeated observations on the stem

and calyx glands, particularly the latter, were required in order to ascertain the true classification of the plants.

Boll glands were counted in an area of 25 sq. mm. located in the center of a valve. Stem glands were counted in an area of the same size located in the middle of the seventh internode below the apex of the main stem. On each plant five boll counts and five stem counts were made, and as many calyx counts as possible, depending on the number of flowers available. Calyx glands were counted in a strip 5 mm. wide, extending from the upper edge of the inner nectary to the rim of the calyx, an area that averaged 41 sq. mm. The use of a low-power dissecting microscope and transmitted light brought to view deeply embedded glands that ordinarily are not visible. In the case of the bolls, the thickness of the wall necessitated removal of some of the soft inner tissue in order to allow passage of sufficient light to illuminate deeply embedded glands.

RESULTS

In 1940, in an F_2 population of 53 individuals that segregated in a 1:2:1 ratio in respect to pitted, intermediate, and smooth boll surface (Smith, table 1),[6] it was observed that the three gland characters mentioned above did not segregate independently of boll surface. The material grown in 1941 enabled the writers to make further observations on the relation of the oil gland characters to the character of the boll surface.

No clear case of dissociation appeared in any of the 97 individuals grown in the F_2 and backcross populations in 1941. Owing to poor development, 4 plants could not be classified satisfactorily in respect to stem glands. More difficulty, possibly resulting from the presence of modifying factors, was encountered in classifying calyx glands, and at the end of the season 11 plants still remained in doubtful status. However, in no instance was a plant classified with certainty as having any combination of the 4 characters other than those that exist in one or the other parent family or in F_1. Therefore, in the absence of positive evidence to the contrary, the 97 individuals in F_2 and backcross populations were classified (table 1) as follows: In 55 plants the 4 characters were intermediate, in 30 plants the characters were associated as in family PH8, and in 12 plants as in family P Hope.

A phenomenal increase in number of boll glands occurs in F_1 and in heterozygous plants in other populations. The mean number of oil glands per unit area in F_1 is 46.4, or approximately the sum of the means of PH8 and P Hope (table 1). Somewhat similar results were obtained in 1940, the counts having been 47.0 for plants with intermediate boll surface, 27.5 for plants with pitted bolls, and 26.3 for smooth-boll individuals. The relatively large number of boll glands in the F_1 is clearly shown in figure 2 (*B*, as compared with *A* and *C*).

The range in number of boll glands per unit area, for each class of all the 1941 populations combined as one array, is as follows: 14 to 29 for plants with smooth bolls, 34 to 58 for heterozygous plants with intermediate bolls, and 23 to 39 for those of the pitted-boll class. The mean number of boll glands in family PH8 exceeds the mean in family P Hope by 2.8 glands (table 1). This difference is only 2.2 times its standard error. However, in view of the ranges in boll

[6] See footnote 3, p. 447.

gland number noted above, the chances are that the smaller number of glands observed in plants having smooth bolls is inherent rather than fortuitous.

The great increase in boll gland number in the heterozygotes is an unusual manifestation of heterozygosity, especially in view of the fact that heterosis is not otherwise apparent in this cross between families that are similar in all but the gland characters. The large number of glands in heterozygous individuals may be explained by assuming that the genes B^p and B^s are complementary in respect to number of boll glands, either gene alone giving rise to only half the number of glands that appear when the two genes are brought together.[7]

The mean number of calyx glands per unit area was found to be 99.9 ± 2.30 in family PH8, 99.0 ± 1.65 in family P Hope, and 96.8 ± 1.54 in PH8×P Hope F_1. Since these populations differed so slightly in regard to number of calyx glands, counts were not made in other populations.

The mean number of stem glands per unit area is given, for each population, in table 1. This character seems unimportant, considering the very small difference (2.4 ± 2.67) between the means of the parent families and the almost identical means in P Hope and PH8×P Hope F_1.

The ratios obtained by Smith (table 1)[8] clearly indicate monohybrid inheritance of smooth and pitted boll surface. The ratios obtained in 1941 are disturbed, but not too seriously, by deficiency of smooth-boll individuals. The values of χ^2 and P given in table 2 are computed from data in table 1. The probability values do not indicate significant departure from the calculated monohybrid ratios.

CONCLUSIONS

The unbroken association of the characters (boll surface, development of the boll glands, size of the stem glands, and size of the calyx glands) that occur only in the combinations existing in the parent families and in F_1, is either an instance of complete linkage or, more probably, a case in which a single gene conditions several phenotypic characters.

TABLE 2.—*Values of χ^2 and P, computed from data in table 1, for the F_2 and backcross populations grown in 1941*

Population designation	χ^2	P [1]
PH8 × P Hope F_2	1.33	0.5
(PH8 × P Hope F_1) × PH8 F_1	.02	.9
(PH8 × P Hope F_1) × P Hope F_1	3.20	.1

[1] The probability (P) values do not indicate significant deviation from calculated monohybrid ratios.

Indisputable evidence of linkage in the genus *Gossypium* appears to be scanty. Harland (*p. 159*)[9] recognizes only three clear cases. Since Harland wrote, McMichael [10] has reported complete association

[7] Acknowledgement is made to J. H. Kempton, formerly of the Division of Cereal Crops and Diseases. Bureau of Plant Industry, for having suggested this explanation.

[8] See footnote 3, p. 447.

[9] See footnote 5, p. 448.

[10] MCMICHAEL, S. C. OCCURRENCE OF THE DWARF-RED CHARACTER IN UPLAND COTTON. Jour. Agr. Res, 64: 477–481. 1942.

of dwarfness and red plant color in Acala cotton, an agronomic variety of *G. hirsutum*. McMichael mentions the possibility of linkage, but nevertheless concludes his account with the following statement: "Indications are that dwarf-red is controlled by a single factor."

In view of the few well-established instances of linkage in cotton, the probability is that the characters involved in the present study are merely different manifestations of a single pair of genes. This conclusion is strengthened by the fact that all of the characters (indirectly, in the case of boll surface) pertain to oil glands.

SUMMARY

Smooth boll surface and three previously undescribed oil gland characters, namely, delayed development of the boll glands, small and deeply embedded stem glands, and small calyx glands, characterize a family of Pima cotton (*Gossypium barbadense* L.) known as P Hope. The contrasting characters, namely, pitted boll surface, early development of the boll glands, large and shallowly embedded stem glands, and large calyx glands, are found in PH8, a representative family of normal Pima cotton.

Observations made in segregating populations of the cross PH8 × P Hope strongly indicate that the characters mentioned above do not segregate independently but, on the contrary, occur only in the combinations existing in the parent families and in F_1. This association of the characters is tentatively accounted for as an instance of manifold expression of a single pair of genes rather than as complete linkage.

Ratios obtained in F_2 and backcross populations agree fairly well with expectations based on the assumption of a single-factor difference. Monohybrid inheritance previously had been found to be the mode of inheritance of pitted boll surface and smooth boll surface, for which the symbols are respectively B^p and B^s. All the above-mentioned characters are intermediate in F_1.

A remarkable increase in number of boll glands occurs in F_1, also in the heterozygous class in F_2 and backcross populations. The mean number of boll glands in F_1 nearly equals the sum of the means that obtain in family PH8 and family P Hope. This is a noteworthy manifestation of heterozygosity, which may be explained by assuming that the genes B^p and B^s are complementary in respect to number of boll glands, either gene alone giving rise to only half the number of glands that appear when the two genes are brought together in the heterozygote.

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